

Prospect of US budget chaos

The US Supreme Court is now to have a say in this year's annual budget charade in Washington. Will it be better able than the Congress to count the cost outside the United States?

THE annual tussle over the US federal budget between the US Congress and the administration will be more confused, even chaotic, this year than ever. That prospect has been on the cards for the past several months, ever since Congress passed the Gramm–Rudman Deficit Reduction Act, with the laudable objective of reducing the federal budget deficit to zero over the next five years, but by the curiously irrational process of delegating control of federal spending to a bunch of accountants at agencies such as the Office of Management and Budget if the Congress and the administration should fail to agree by deadlines specified in the act. That the Congress, which owes many of its powers over the federal budget to the eighteenth century doctrine of “no taxation without representation”, should have been willing to delegate this central function as specified by the act was one of the big surprises of last year. That President Reagan should have been willing to let his hands be tied was an even greater puzzle, although the budget published last week suggests that he saw it as a chance to launch a budget free from spending of which he disapproves. The bombshell that has thrown all these calculations into disarray is the decision of a federal court last week that the Gramm–Rudman act is unconstitutional. The US Congress is to appeal to the Supreme Court, hoping that there will be some kind of decision by the early summer, in May or thereabouts. What this means is that the fierce debates about the budget that will occupy the next few months will be conducted in a vacuum. Political and economic chaos have supervened.

Last week's budget is hardly the place from which rational discussion could in any case have begun. Although the budget as a whole complies with the Gramm–Rudman act by planning to spend just less than the limit specified for the financial year beginning on 1 October, the President's package (see p.526) flies in the face of known congressional opinion. Defence spending is increased, most other forms of spending are reduced. Although basic research fares relatively well under the proposals, it is a striking measure of the skew of the package that direct support of research by civilian agencies should, under the plan, amount to only a half of what the Department of Defense would be spending on military research. In a normal year, the Congress would now embark on the familiar process of writing back into the budget items on which it considers that money should be spent; no doubt it would also repeat last year's process of cutting back the defence budget. The possibility that the Gramm–Rudman act may be unconstitutional will give heart to those who think that this year will be no different from previous years. So the result is likely to be that, by the deadline of 15 August specified under the act, Congress's version of the budget will far exceed the limit of \$144,000 million. What will happen then?

Devastation

One of three things. If the Supreme Court finds the act to be constitutional, its provisions will come into effect, enforcing automatic reductions on rather less than a third of the total of planned expenditure. The effects could be devastating for some important programmes, those of agencies such as the National Science Foundation, for example. So financial chaos by the

summer is one possible outcome. But the US Congress will not take well to such a prospect, and might decide that the best way of dealing with Gramm–Rudman is to get rid of it, in which case the whole episode will be seen in retrospect as having been another budgetary charade. That is the second outcome, little different from the third now in prospect that the Supreme Court will find the act unconstitutional, which will be a signal for a return to the budgetary process that has become familiar and notorious in the past decade. In theory, of course, the Congress might throw in the towel and swallow the substance of the administration's package for the sake of good government. But not in an election year.

Chaos

Where will it all end? And why, in any case, should it matter? The immediate effect of the annual confusion about the US budget is that the agencies depending on it for their spending authority do not know where they stand from one month to the next. The effects on the basic research agencies are to impede forward planning and thus the rational use of resources. That condition, now chronic, will be made worse this year. The effects of this uncertainty on the economy of the United States, and on its external monetary relations, may be even more serious. Only a few weeks ago, representatives of the five largest industrial economies meeting in London appear to have agreed that a concerted attempt to reduce money interest rates would make sense. But the capacity of the United States, which has a central influence on worldwide interest rates, to play a part in any such campaign is hamstrung by the federal deficit, which has to be financed by means of a competition for funds on the money markets of Wall Street. If it had seemed that Gramm–Rudman would have worked, it would at least have been possible to anticipate a less fierce competition. Now, with the whole issue clouded by uncertainty, the money-people will assume that the United States will remain a heavy borrower for at least the next two years. Economic growth will be held back in prosperous countries. Those, like Mexico, teetering on the edge of collapse because of a burden of foreign debt will look even more apprehensively at the future.

This is too high a price to pay for the US government's long-standing failure to agree on a mechanism for deciding how much the administration should be allowed to spend on what. The difficulty is only partly constitutional, although the convention that, in budgetary matters, the President proposes and the Congress disposes provides the opportunity for the familiar chaos. The more serious difficulties are political, stemming from the perennial conflicts between successive administrations and the Congress in questions such as the necessary cost of defence, the importance of social programmes of various kinds and the devotion of individual members of the Congress to local spending projects that may enhance their chances of re-election. What this year's budget cycle shows is that neither party to the exercise has an incentive to compromise with the other. The Gramm–Rudman shotgun will clearly not do the trick. Maybe an arrangement that no member of the Congress should be paid a salary until the budget has been agreed would be more effective. □



World Archaeological Congress

South African exclusion causes academic schism

REPORTS to the contrary notwithstanding, the World Archaeological Congress will take place this September in Southampton, England, without the participation of South African and Namibian scientists, but it will not enjoy the blessing of the International Union of Prehistoric and Protohistoric Sciences (IUPPS), which will instead recognize a congress at Mainz, West Germany, as its official quinquennial meeting.

At a meeting of the British executive committee for the Southampton congress at the British Museum, organizing secretary Professor Peter Ucko said plans for the congress will go ahead. But three of the five members of the executive committee, Sir David Wilson, Professor Leslie Alcock and Professor Colin Renfrew, resigned, saying they could no longer officially support the Southampton congress.

IUPPS had threatened to withdraw its support for the meeting unless the British organizers lifted their ban on South African and Namibian participation. After a meeting in Paris last month, the executive committee of IUPPS gave the British organizers until 15 February to reinstate the South Africans. The executive committee also insisted on written guarantees that groups opposing South African participation would not disrupt the congress.

For the past three weeks, organizers of the Southampton congress have been trying to work out a formula that would allow them to comply with the IUPPS demands. One compromise proposal would have been to seek approval of South African participation from the groups opposing it on a one-time-only basis. Once the Southampton congress received IUPPS approval, a motion would be put forward to ban South African scientists from any future meeting until South Africa's apartheid policy was lifted. But in the end, this compromise fell through, and Ucko was forced to acknowledge that he could not meet the IUPPS requirements.

According to Professor John Evans, the issue of South African participation might never have come up but for the attempts by meeting organizers to broaden the focus of the Southampton meeting. The last IUPPS international meeting was held in Mexico, the first time ever outside Europe, but hopes for a truly international meeting at that time were not realized. In trying to attract wider participation by nations outside the industrialized West, the issue of South African participation was thrown into stark relief.

Evans resigned last month as president

of the executive committee of IUPPS and the British executive committee because he too did not want to be associated with a congress held without IUPPS endorsement (see *Nature* 319, 251; 1986).

The Southampton meeting organizers now find themselves in an extremely difficult position. Encouraged initially by IUPPS to provide a broader scope to the Southampton congress by doing more to encourage participation of developing countries, they saw banning South African scientists as their only alternative. But having succeeded in attracting some formerly neglected parts of the world archaeological community, they now find they have alienated many mainstream archaeologists. Ucko estimates that the Southampton congress will attract only 1,000 of the 3,000–4,000 scientist who might otherwise have attended if IUPPS support had not been withdrawn.

Ucko maintains that the IUPPS execu-

tive committee has exacerbated the situation. At its January meeting in Paris, the committee refused to consider the positions of members from developing countries who could not be present. Ucko considers their actions "utterly scandalous", claiming the committee was only too happy to move the congress to Mainz in order to maintain the stranglehold of Western European scientists on the world archaeological community.

Opponents of the South African ban say it is an issue of academic freedom. They maintain that no *bona fide* scientist should be excluded from participating in a scientific congress for political reasons. Nobody can oppose the concept of academic freedom, says Professor Colin Renfrew, but blindly adhering to it is pointless if it causes *de facto* discrimination. In this case, including South Africans would mean not including Indians and Nigerians, among others, who have said they will not attend if the South Africans do. "I see no solution to this dilemma", says Renfrew.

There is no question that the issue of the Southampton congress has caused a split in the archaeological community. Says John Evans, "a certain amount of damage has been done, and it will take time to repair it".

Joseph Palca

Animal welfare

Tip-off leads to NIH ban

Washington

THE National Institutes of Health (NIH) are getting tough about enforcing animal welfare rules. NIH's Office for Protection from Research Risks has started making unannounced visits to check conditions in animal laboratories using Public Health Service funds, and as a result has suspended NIH funds to the Health Sciences Division of Columbia University in New York.

The order against Columbia was made by NIH director James Wyngaarden after he received an interim report from a team that visited Columbia's Health Sciences Division on 23 and 24 January. The report identified deficiencies in four areas: veterinary care, sterility of areas where animals recuperate from surgery, housing of dogs under quarantine and methods of reducing health risks to personnel. The suspension affected only NIH funds used for research on vertebrates other than rodents, but the university has voluntarily halted all other research until the facilities are improved.

The new get-tough policy at NIH came into force formally at the end of last year, when the Public Health Service introduced new regulations that significantly tighten the requirements on grantee institutions that conduct animal research. Many of what were previously suggestions relating to animal use committees became

mandatory requirements: a non-scientist must be included, and the committee must review all research proposals involving animals, to name but two. In addition, institutions were required to submit reports to NIH detailing their arrangements for meeting the new regulations. Columbia University submitted its report well before the 31 December deadline and made its failings plain, although the university had as long as a year ago been aware that "considerable improvements" were necessary and had instituted a long-range plan to achieve them. NIH were tipped off to investigate Columbia by an animal welfare group, and will be ready to listen to similar tip-offs in future.

The research that is now on hold at Columbia involves rabbits, dogs, sheep and primates. Research topics include cardiovascular disease, fertility, fetal development, immunology, birth defects (including Down's syndrome), and arthritis.

Other unannounced inspections can be expected, and NIH is not being shy about enforcing its conditions. NIH funds to the City of Hope Medical Center in Duarte, California have been suspended, and the University of Pennsylvania is barred from new NIH research funds pending agreement over requirements; head injury research at the university was suspended indefinitely last summer following discovery of serious abuses. **Tim Beardsley**

European particle physics

CERN budget scrutiny planned

AN international review of the European Organisation for Nuclear Research (CERN) seems certain to go ahead this year. The result of a British initiative, the review would consider the impact on CERN of possible reductions in contributions by the 14 member states. The British have tabled a resolution to set up the review, a proposal that is likely to be adopted by CERN's council at an extraordinary session on 20 February. The British move is a first step towards implementing the recommendations of its Kendrew committee, which advocated last year that Britain should seek a cut of 25 per cent in its budget contribution or withdraw from CERN.

Short of complete withdrawal, the review is the only way that Britain may eventually reduce the cost of its membership, now six per cent of the total budget of Britain's research councils. The panel, chaired by Sir John Kendrew, concluded last year that this proportion is too high, and said that the reduction of the British contribution to CERN should be attained by 1991. The Science and Research Council (SERC) and the Advisory Board for the Research Councils subsequently endorsed this position, since when George Walden, a junior minister at the Department of Education and Science, has been trawling the capitals of Europe, testing the waters for a concerted bid to reduce the cost of CERN.

The outcome seems to have been that European ministers have agreed that it would be unobjectionable if CERN were cheaper, and that possibilities for making it so should be looked at. The council of CERN consists of ministerial representatives who are not necessarily high-energy physicists and, despite earlier misgivings, the British government is now persuaded that the council is the appropriate body to establish and monitor an independent inquiry.

The British draft resolution suggests that the review should encompass European high-energy physics and CERN, and that the effects of cuts of up to 25 per cent in CERN's budget should be assessed. While possible names for the panel — such as the director of a large German laboratory and senior industrialists — are being considered, the draft resolution leaves this aspect open. The somewhat optimistic intention is that the panel should be established within a month and should report by the end of the year. Presumably Britain would then be obliged to follow the recommendations of the panel or withdraw.

One obstacle to a reduction in CERN's funds may be the organization's success in keeping the budget as tight as it is. The

construction of CERN's major new accelerator LEP (a large electron — positron collider) has been carried out within a budget that has been constant in real terms, and which will not increase at least until experiments begin in 1989. The result has been the closure of one major facility, the Intersecting Storage Rings, and the delayed refurbishment of other facilities and repayments of loans. After LEP is finished, these commitments will become due. While there may be scope for managerial change and contracting out of design or construction work, it is hard to see how a cut of 25 per cent could be achieved without devastating CERN's scientific capability.

Another obstacle to cuts is US competition. The Stanford Linear Collider (SLC) is expected to come on line in 1988 (which some CERN physicists think optimistic) and will examine, though with a lower particle production rate, some of the same phenomena as LEP. These include the production and decay of *W* and *Z* bosons. One particular goal that SLC could reach before LEP is the cataloguing of the ways in which the *Z* boson can decay, which should reveal, for instance, whether there are more than the three types of neutrino already known, a result that may have repercussions for cosmology as well as for particle physics. SLC may also find the "top" quark, whose possible detection at CERN last year is not yet confirmed.

A more difficult goal, which SLC almost certainly will not achieve, is the observation of the Higgs boson, a particle associated with symmetry breaking within the unified theory of electromagnetic and weak interactions and of potentially crucial importance in understanding how particles come to have mass. Higgs bosons may be produced by the decay of only one in every 100,000 *Z* bosons, which is why LEP's higher particle interaction rates should be crucial.

These and related phenomena would be much better investigated were LEP to achieve its full design capability by the incorporation of superconducting magnets around the 30 km circumference accelerator ring. This would permit centre-of-mass collision energies of 200 GeV to be achieved, enough to produce pairs of *W* bosons, for example. But it seems unlikely that the 200 million Swiss francs required could be found without an increase in the CERN budget. Perhaps this will hang on decisions about the proposed US Superconducting Supercollider, an ambitious scheme that seems unlikely to survive unscathed the current US budgetary problems.

Further ahead, there may be scope for savings at CERN in the mid-1990s, when a

significant proportion of CERN staff are due to retire (at 80 per cent of their salaries). But since one of Britain's principal aims in setting up the Kendrew panel in 1984 was to find money for its hard-pressed "small science" community, that prospect may be too distant. SERC has already decided to cut its domestic high-energy physics budget by 20 per cent by 1991, losing 150 of the 750-strong community. Whether there will be such economies at CERN will not be known for at least a year.

Philip Campbell

AIDS protein made

Washington

GENENTECH, the Californian biotechnology company, last week announced the synthesis in mammalian cells of a recombinant protein that is the favoured candidate for a vaccine against acquired immune deficiency syndrome (AIDS). Although the work marks an "unexpected modest advance" in AIDS research, it may greatly expedite the search for a safeguard against a disease that has already claimed more than 8,000 lives in the United States.

The protein, gp120, is part of the envelope of the AIDS virus, HTLV-III or LAV. Although it has been synthesized by several laboratories in bacteria and yeast cells, these systems cannot match the mammalian cell products, which Duke University's Danni Bolognesi calls "as close to the real beast as you can possibly get". Genentech's Chinese hamster ovary (CHO) cells glycosylate and secrete the protein, while microbial cultures must be engineered to do either. Despite the added expenses of maintaining mammalian cultures, Genentech's system may prove more efficient in the long run because it cuts purification costs.

Located on the viral envelope, where antibody recognition is thought to occur, gp120 has long been the focus of interest for those looking for an AIDS vaccine, but has been a devil to isolate from the virus itself. So, before Genentech's team brought its efforts to fruition, obtaining large quantities of the protein in its natural glycosylated form was both dangerous and laborious. Now senior scientist Larry Lasky and Genentech's collaborators can hasten vaccine testing, which they have just begun with rabbits, guinea pigs, rats and mice.

Genentech tried the same genetic tricks that produced its herpes vaccine last year to coax the AIDS virus protein from hamster cells; what those tricks are, the company is not saying. Although Lasky seems pleased with his latest sleight of hand, he is also cautious. As a vaccine, gp120's efficacy has yet to be proved, and it may be a year or two before monkey trials provide any data relevant to the human response. Any possible vaccine will also have to counter the many variant forms the AIDS virus is known to assume. Karen Wright

US budget

Biosciences lose out to defence

Washington

A HUGE increase in defence spending, including defence research and development, dominates President Reagan's proposed budget for fiscal year 1987. Total federal obligations for research and development, including facilities, are estimated at \$63,000 million, 16 per cent above the 1986 level of \$54,000 million. Military programmes in the Departments of Defense and Energy, as in previous years, account for most of the increase. But substantial increases are also proposed for the National Science Foundation (NSF) and the National Aeronautics and Space Administration (NASA). The physical sciences in general have been relatively well treated. Biomedical research is a different story.

The budget continues all the well-known Reagan themes. Budget authority for the Strategic Defense Initiative (SDI) will increase from \$2,800 million in 1986 to \$4,800 million in 1987, Congress permitting. Research and development by the Department of Defense would increase to 60 per cent above its 1984 level; only \$21,000 million of the \$63,000 million budget for federal research and development would be non-defence, \$7,600 million of that for non-military basic research. Pet Reagan hates such as the Sea Grant programme of the National Oceanic and Atmospheric Administration and the computer sciences and technology and fire and building research programmes at the National Bureau of Standards are scheduled (as they have been before) to be killed. The Environmental Protection Agency takes a 7 per cent cut.

Tension between the administration and Congress over the budget is unusually high this year, because of last year's controversial legislation setting fixed targets for reducing the federal budget deficit in each of the next five years. Under the Gramm-Rudman deficit reduction act, automatic across-the-board cuts will come into effect if Congress fails to pass budgets meeting the target.

The new 1987 budget proposal foresees total spending of \$944,000 million, with a deficit of \$143,600 million, just under the limit specified by Gramm-Rudman. But the administration's refusal to consider tax increases, together with a 12 per cent increase in total military spending, has meant hefty cuts to many domestic programmes, which Congress is unlikely to approve. Instead, the appropriate committees and Congress as a whole can be expected to make several changes, with the result that the actual budget for the financial year beginning on 1 October 1986 will probably look even less like the President's proposal than usual, if

Gramm-Rudman stands. But further uncertainty was introduced last week when a federal judicial panel unanimously ruled the automatic cuts provision of Gramm-Rudman unconstitutional; the question will now go to the Supreme Court, which is not expected to rule before the summer.

Meanwhile, the administration's proposals are as follows.

National Science Foundation

The foundation's budget request is \$1,685.7 million, an increase of 8.4 per cent. Even so, the mood at NSF is temperate. Director Erich Bloch points out that in 1983 constant dollars, the 1987 request represents a negligible increase over the 1985 amount (after a decrease in 1986). The foundation says that even if Congress should approve the full request, that would be only just good enough, in part because of the increasing complexity — and cost — of research equipment. But in the Gramm-Rudman era's scramble for funds, full approval seems unlikely.

NSF lists for "special emphases" for 1987: biotechnology, computational science and engineering, global geosciences and broadening participation in research and education. If NSF sustains budget cuts during the impending legislative fray, the foundation would probably seek to protect them.

NSF is planning to establish between two and four new multidisciplinary biotechnology research centres, as well as an undefined number of so-called mini-centres. A 50 per cent increase to \$84 million is wanted for computational science and engineering; the global geosciences effort would jump from \$17 million to \$35 million, and would include the development of Earth-based and satellite observational instrumentation, a project for which the administration has a special enthusiasm.

NSF's plans to expand participation in science and engineering research and education include \$4 million for the new Minority Research Centers of Excellence programme and a \$13 million increase

Due process of law

IN anticipation of legal challenges to the constitutionality of Gramm-Rudman, the bill itself contains specific procedures to be followed. Rather than proceed from district court to appeals court to Supreme Court, any challenge goes first to a special panel of federal judges who issue a ruling. If there is an appeal against the panel's ruling, the case goes immediately to the Supreme Court.

In fact, challenges did arise. Several congressmen joined a suit brought by the National Treasury Employees Union. On 7 February, the panel issued its ruling declaring the automatic-cuts provision of Gramm-Rudman unconstitutional.

Now it falls to the heavily burdened Supreme Court to make a final determination. That decision is unlikely to come before July. Until then, federal agencies will have to live in fear of next fall's Gramm-Rudman axe. □

over the \$50 million spent in 1986 to improve undergraduate research.

Two more of the agency's pet projects are seeking increased support. NSF's supercomputer centres stand to gain almost 19 per cent over their current \$45.2 million, but the total, \$53.6 million, will be spent on bringing on stream the five centres now being set up. NSF has chosen to push networking and software research rather than to establish new centres in 1987, even though the National Science Board's initial mandate called for 10 facilities.

The nascent engineering research centre network grabbed a \$12 million increase over this year's budget of \$23 million. Six centres have been established so far, and NSF hopes to have 15 by the end of 1987.

National Institutes of Health

The proposed total budget for the National Institutes of Health (NIH) is \$4,936 million, a substantial drop from the \$5,353 million comparable figure appropriated by Congress for 1986. This repeats a now familiar pattern: the administration proposes cuts for NIH, only to have Congress reverse them and vote huge increases a few months later. But this year, because of

US research and development budget (\$ million)

	1985 actual	1986 estimated	1987 proposed
Defence-related	31,099	33,485	41,823
Health and Human Services	5,444	5,524	5,471
(NIH)	(4,824)	(4,905)	(4,672)
Energy	4,901	4,785	4,886
NASA	3,235	3,594	4,051
NSF	1,346	1,334	1,508
Agriculture	941	922	907
EPA	320	334	310
R&D facilities	1,894	1,812	1,734
All other	2,205	2,048	1,847
Total	51,385	53,836	62,537



NASA's air-breathing hypersonic "Aerospace Plane".

the Gramm–Rudman Act, there is less confidence that Congress will come to the rescue, and NIH officials are acting "as if they really think it's going to happen", according to one congressional observer.

In addition to the sharply lowered budget for 1987, the administration is proposing a rescission to the NIH 1986 budget of \$62 million. This is on top of \$230 million that is to come out of the 1986 budget because of automatic Gramm–Rudman cuts this year. But the \$62 million rescission will occur only if Congress specifically approves it within 45 days, which must be considered doubtful.

Most of the savings sought at NIH would be achieved by limiting the total number of extramural research grants to 18,000 — 776 less than Congress intended for this year. By focusing on the total number of grants, NIH hopes to shift the debate from the number of new grants awarded each year, which has been a continuing battle with Congress. But most congressional sources last week expressed incredulity that the proposed NIH budget could be taken seriously; most probably, there will at least be some restoration of the budget in the direction of last year's figure.

If not, the number of new and competing extramural grants will be 5,519 this year (6,100 had previously been agreed); this number would decrease steadily to the administration's long-sought target of 5,000 in 1989. In the short term, the rescissions may mean that NIH will have to reduce the dollar value of research grants.

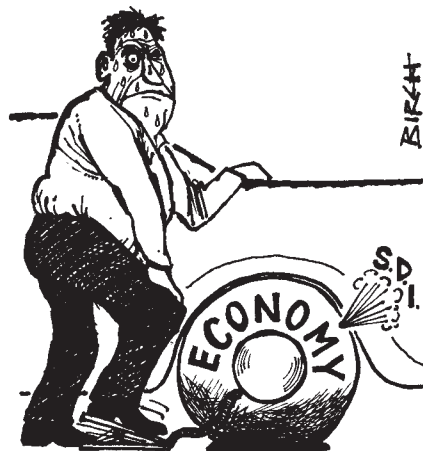
A total of \$213 million is proposed for research into and control of acquired immune deficiency syndrome (AIDS), to be determined as a special fund in the office of the assistant secretary for health. A sum of \$46 million would go to the Centers for Disease Control, \$144 million to NIH, \$14 million to the Alcohol, Drug Abuse and Mental Health Administration and \$10 million to the Food and Drug Administration. The total appropriated for AIDS research in the current year was \$244 million, but the combined effects of Gramm–Rudman and the proposed rescission would reduce that to \$193 million.

Compared with the 1986 planned allocations, relatively more AIDS funds would go to NIH (an increase of \$22 million) than to the Centers for Disease Control (which would get \$5 million less).

Aeronautics and Space

The budget requested for the National Aeronautics and Space Administration

(NASA) is \$7,694 million, a 5.3 per cent increase over the estimated 1986 budget after Gramm–Rudman cuts. Nevertheless, acting NASA administrator William Graham preferred to look at it another way last week: the 1987 total is still a reduction in real terms of 3.5 per cent from the level of the 1986 operating plan. In any event, NASA's budget is completely up for grabs again now with the loss of Challenger; NASA will save on shuttle operations until flights resume but will also lose part of the \$704 million it had been expecting in reimbursable expenses from shuttle operations in the current year. The 1987 budget included \$410 million for President Reagan's space station, the least that is plausible if the space station is to be in orbit by 1994 as the President has directed. The budget also included \$29 million to "initiate" TOPEX, an oceanographic satellite being developed jointly with France. Officials declined to speculate on whether supplementary funds will be sought to build a Challenger replacement (cost estimates range up to \$2,900 million), but legislation has already been introduced in the House of Representatives



that would do just that. NASA already has spare parts in storage that could form the basis of new orbiter to be built quickly.

About \$200 million is requested for a joint project to be carried out by NASA and the Department of Defense to develop a new class of hypersonic aerospace vehicles capable of horizontal take-off and landing on normal runways. The National Aerospace Plane, as it has been dubbed, would be mainly air-breathing and would use ramjets with hydrogen fuel, operating at speeds of between Mach 12 and Mach 25 at altitudes of up to 350,000 feet. The plan is to have a test model in the mid-

1990s. The idea has been developed over the past several years by NASA and the Defense Advanced Research Projects Agency; all participants believe there is still "a high degree of technical risk". The planes would serve both civilian and military uses; ten minutes from the east coast of the United States to the west coast was one claim bandied about last week. Perhaps less frivolously, the administration may be attracted to the idea of being able to deploy US forces anywhere in the world within a few hours.

Energy

The request for the Department of Energy (DOE) in 1987 is only about 1 per cent above actual spending, a decrease in real terms. But within this total there is a 11 per cent increase for the department's nuclear weapons production and support (to \$4,830 million), and a 7 per cent decrease for research and development (to \$4,539 million).

As in previous years, cuts of 50 per cent and more are proposed. Magnetic fusion research is also scheduled to decrease, from \$365 million to \$333 million. Fossil energy research is scheduled for major cuts, but there is a new clean coal technology fund for which \$150 million is requested in 1987 (1986: \$100 million) and a new cooperative research and development venture pool with industry is proposed. The aim is to get industry to pay for much of the research on fossil fuels traditionally supported by DOE; if successful, conservation might be handled in the same way.

Fission research for civilian reactors (including breeder reactors) is to be phased down, while nuclear space and defence power systems (including SDI) increase from \$20 million to \$71 million. But high-energy physics and nuclear physics both get a boost, with first-time operating funds for the Tevatron collider at Fermilab and for the Stanford Linear Accelerator. Low level development funding continues for the Superconducting Supercollider; a decision on whether to go ahead is expected this year, and budget allocation for high-energy physics is suspended until then. Funds are requested to initiate construction of the Continuous Electron Beam Accelerator Facility at Newport News, Virginia.

Agriculture

The agricultural research service does well, with a 7 per cent increase to \$497 million. The service conducts basic research in plant germplasm and biotechnology. But the cooperative state research service, which deals with more local problems, will be cut by more than 15 per cent; the administration believes local problems should be researched by state governments. The animal health and disease programme would be eliminated; in previous years Congress has reinstated it.

Tim Beardsley & Karen Wright

Japanese research

Small increase brings big gains

Tokyo

OVERALL support for academic research in Japan will not keep pace with inflation this year, according to the 1986 budget figures of the Ministry of Education, Culture and Science. The ministry is by far the largest public source of support for basic research in the universities, but it has won only a tiny overall rise (less than one per cent) in funds for the direct support of research and international exchange.

The ministry's figures compare badly with the boom in spending on research by industry, a boom that puts Japan second only to the United States in total research spending in the non-communist world. The root of the problem lies in the high priority given by the government to tack-

Japan's science budget (contd)

	Yen (thousand million)	Percentage increase or decrease
Ministry of Education, Culture and Science		
Grants in aid of research	43.5	+3.5
Maintenance of scientific research system	7.8	+36.8
Research fellowships	0.8	+41.7
Energy research	20.8	-25.2
(Nuclear fusion)	(8.2)	(+9.2)
(Accelerator physics)	(5.9)	(-58.2)
Space research	12.4	+12.9
Marine science	0.5	+41.8
Earth science	2.0	+1.0
Polar research	3.1	-7.1
Cancer research	1.8	+6.0
International academic exchange	3.7	+1.9
International student exchange	11.7	+16.2

ling the ever-increasing budget deficit and the austere budgets this makes necessary.

Although little has been gained overall, there are some bright spots. Direct grants-in-aid of research are up by 3.57 per cent, an annual rate of growth that has been fairly constant over the past four years, and which is ahead of the rate of inflation (about two per cent during the same period). Overall, 15,000 grants were given out, from 53,000 applicants.

Big grants tend to fall into two categories: those for "special distinguished research" likely to produce outstanding results recognized internationally, and those for "special research". The latter is an unusual category in that it is awarded to a field rather than to an individual; researchers all over Japan may apply to participate in such a project. Both types of grants go to scientists at the leading edge of research and their topics give a good idea of what are perceived as the hottest areas. As elsewhere, molecular biology and semiconductor physics are currently among the winners.

Among the 13 special research projects (total cost ¥5.3 thousand million, US\$26.5 million) beginning this year are those on the regulation of complex genetic

systems and their responses to stresses in the intracellular and extracellular environment, the physiology of blood vessel endothelial and smooth muscle cells and changes in arteriosclerosis, biologically active substances studied through chemical synthesis and the development of biosensors in the "special distinguished" category, projects (total funding ¥2.3 thousand million) have recently begun on semiconductor superlattice surface structure, the quantum Hall effect and compound semiconductor/insulator interface physics. On the biological side are those on B-lymphocyte multiplication and differentiation, the mechanism of division of animal cells, the regulation of vasoactive peptide synthesis, the regulation of enzyme synthesis in higher animals and a new protein synthesis system.

Overall, though, there has been no increase in total support for these top grants. The increase in grants-in-aid is concentrated in the smallest category of grants (up to ¥3 million) available for individual projects, the small grants (up to ¥1.2 million) available for researchers under 37 and the grants for cooperative research between different institutes.

Extra money has gone towards strengthening the "scientific research system" (up 36.8 per cent). Most of the increase goes towards a restructuring of the Tokyo University bibliography centre as a nationwide scientific information centre.

There is also a big increase in funds for postdoctoral fellowships, although the

original amount being spent was not large.

Many of the increases are being paid for by cuts in the budget for energy research including a huge reduction in funding (-58.2 per cent) for the TRISTAN project, the construction of an electron-positron collider ring at the Institute of High-Energy Physics in Tsukuba Science City. The ring is now almost complete and will be commissioned in November, at energies around 23-25 GeV. With the addition of superconducting cavities, it should be possible to reach 36 GeV, but plans to turn the whole ring into an electron-proton collider have virtually been abandoned.

One ambitious project that does seem likely to bear fruit is the scheme developed by the Institute of Space and Astronautical Science (ISAS) designed to put a satellite into orbit around the Moon. Following the success (so far) of the first deep-space launch that sent the satellite *Suisei* towards a March encounter with Halley's comet, funds have been obtained to work on a prototype for a Moon launch (see *Nature* 315, 173; 1985) in the early 1990s.

Another ISAS project will be supported from the funds set aside for international cooperation — several years of discussion with Chinese scientists have resulted in a high-altitude balloon launch programme which begins with a maiden flight in July.

Marine science also seems to receive a boost (+41.8 per cent). But most of this is to pay for the expensive business of full participation in the Ocean Drilling Program, even though the drill ship is not expected in Japanese waters until the 1990s.

Alun Anderson

Gorbachev's purge reaches researchers

THE Communist Party of the Soviet republic of Kirgizia has accused the Kirgiz Academy of Sciences of "serious failings". Addressing the Party Congress last month, the First Secretary of the Kirgiz Party, Absamat Masaliev, accused the academy of publicizing even routine and insignificant developments as major breakthroughs, and even reporting research work that had not been done at all. At the previous party congress, five years ago, the president of the academy, Murzabek Imanaliev, had claimed that its scientists had, "for the first time anywhere", obtained "natural iron powders, alloyed with rare earth elements", and had established with industry a semi-pilot plant for the production of such powders. This claim, Masaliev said, was simply wishful thinking.

To bolster such claims, Masaliev continued, the Kirgiz academicians have spent considerable sums on organizing symposia and conferences, and have bolstered their prestige with lavishly furnished offices. Yet the scientific standard of the Kirgiz

academy is so low, he said, that not a single institute or team has been included in the new "inter-branch" scientific-technical complexes now being established union-wide, as part of the "scientific-technological revolution".

This is not the first republic academy to be attacked for slackness in the run-up to the All-Union Party Congress later this month. A few weeks ago, the Uzbek academy was accused of nepotism and general slackness, attributed to the prolonged illness of its president, who has since been replaced.

But the charge of falsifying research claims is far more serious. It is standard Soviet practice to embark on a major shake-up or purge by selecting a few smaller bodies as examples, thus giving more important institutions a chance to put their affairs in order without public censure. Indications are that the programme of reforms launched by Mr Gorbachev is taking a close look at research as well as the administrative sector.

Vera Rich

Pharmaceuticals in Britain

Drug list clouds foreign interest

DESPITE the recently announced decision by the Swedish pharmaceutical company Astra to develop a £2.5 million research facility in London, the climate for investment in the United Kingdom by non-British pharmaceutical companies remains poor. The chilly conditions are caused by the industry's lingering irritation over the "limited list", a scheme introduced by the British government over a year ago that determines which drugs the National Health Service (NHS) will pay for.

Pharmaceutical companies are still smarting from what they feel was an arbitrary decision to implement the limited list that effectively blacklisted certain drugs, and cut deep into profits.

Astra's plan to support the Neuroscience Research Centre at the Institute of Neurology, a part of the University of London, is a positive sign, but it is probably not the harbinger of a flood of new investments.

Before the introduction of the limited list, investment by industry in UK facilities was enjoying a fairly rosy period. The US company Merck Sharp and Dohme had established a £25 million Neuroscience Research Centre in Terling Park near Cambridge, the Swiss giant Sandoz had put up £7 million for a research unit with a staff of 50 at University College London and Parke Davis, the pharmaceutical division of US giant Warner Lambert, was building a £4 million facility on campus at the University of Cambridge.

Another US company, Wyeth, had "pens poised" to sign agreements for a £30 million research unit in Swindon, while its rival A.H. Robbins had just completed a new production facility at Langherst in West Sussex that was to be expanded to include a research facility.

But when the limited list was announced, the cries of anguish from the drug companies could be heard around the world.

Wyeth cancelled its plans for Swindon, Robbins sold off its Langherst plant, and Parke Davis made it abundantly clear that it would never have gone into the United Kingdom if it had known about the government's plans.

G.D. Searle also insisted that the closure of its High Wycombe facility was prompted by government pricing schemes, although corporate reorganization following the Monsanto takeover of Searle probably also played a major role (see *Nature* 319, 251; 1986).

British-owned companies, by contrast, have come through the limited list ordeal nearly unscathed. On the eve of the public sale of 25 per cent of Wellcome plc, the

company reported its most successful year ever, although its rate of profit on British sales was considerably less than those in other parts of the world. Glaxo, too, has done well, and was able to step in to buy Robbins' Langherst production plant. According to Glaxo research director Alan Williamson, "the climate for research and development efforts in this country is still very good".

Glaxo's impression is hardly shared by foreign pharmaceutical companies. E.J. Fullager, UK chief executive of Sandoz, says the climate is still one of "uncertainty,

writ large". Unless things deteriorate substantially, Fullager says, Sandoz will continue to support its institute at University College, but he admits that Sandoz has been "disappointed" by recent government actions.

The government is aware of the disaffection it has engendered by the limited list. Health Department officials now say they are negotiating with the Association of British Pharmaceutical Industry (ABPI) on revisions to pricing schemes, "so that the industry might be offered a period of greater stability in its sales to the NHS".

But a spokesman for ABPI says "it will take a long time for the industry to forget what happened".

Joseph Palca

West German forests

Deterioration, but some recovery

Munich

THE 1985 Forest Damage Inventory, the results of which were published at the end of last year, shows only a slight deterioration of West Germany's forests since the previous year. It is now estimated that 52 per cent of the total forested area is affected by the "new type forest decline", compared with 50 per cent in 1984 and 34 per cent in 1983. Damage increased at higher altitudes and notably in the Alps, but there were also the first signs of recovery in some lowland areas.

The latest inventory of forest damage is the fourth of a series of annual nationwide damage assessments initiated by the federal government but carried out by the regional governments, the *Länder*. Only the 1984 and 1985 inventories are based on a comparable assessment and classification method. Assessment areas are defined by the nodes of a 4 × 4 km plot, where a specified number of trees is classified into five damage categories ranging from category 0 (undamaged) to 4 (dead). Damage classification is based on the extent of loss of needles or leaves, with the discoloration of foliage as a second criterion.

The much disputed damage category 1 (loss of needles and leaves between 11 and 25 per cent, compared with a reference tree) is for the first time being referred to as a warning stage rather than a clear damage stage. Concentrating on the subsequent damage categories 2 to 4, the overall change between 1984 and 1985 has been a slight increase from 17 per cent to 19 per cent of the total forest area.

Broad-leaved trees such as beech and oak are now showing the same degree of damage as conifers, which were first affected by the disease. In particular oak has deteriorated markedly, with 16 per cent of oak forests being classified as medium to severely damaged.

As in previous years, there is still a dis-

tinct north-south gradient, with the southern *Länder* of Bavaria and Baden-Württemberg most severely affected. There is now also much concern for the alpine forests, where damage increased from 34 per cent in 1984 to 53 per cent in 1985 (damage categories 2-4 only). The West German concern for highland forests is shared in Switzerland. The Swiss 1985 "Sanasilva" forest damage inventory revealed marked damage at higher altitudes compared with lowland forests; for forests at an altitude of 900 m or more, 11 per cent of all trees assessed were medium to severely damaged (the Swiss damage classification is comparable to the West German). This is seen as a major threat to villages and roads, which are often protected by forests against avalanches and floods.

In contrast with the deterioration of highland forests, there is a slight recovery of some lowland forests in Germany. There is also a slight overall recovery of pine, with a decrease in the medium to severe damage from 21 per cent to 17 per cent. This local recovery, and the relatively small increase in the overall damage when comparing the 1985 results with those of the previous inventories, is largely attributed to favourable climatic conditions.

The causes of the decline are still open to debate. Regional differences in the symptomatology and the time-course of the disease have led to the suggestion that the forest decline may not be a single disease, but a series of different disease types.

These disease types may have different causes, but there is probably a common synchronizing factor triggering their onset. As regions of different pollutant levels are equally affected, it seems plausible that this synchronizing factor is an overall climatic event such as drought or frost.

Lutz W. Blank

Eureka

Secretariat in search of base

PROJECT Eureka continues to make progress towards its goal of promoting European cooperation in high technology projects, but like most multinational cooperative ventures, some steps are disappointingly slow.

On the positive side, the Eureka High Representatives at their meeting in London late last month announced that new collaborative efforts are making "significant progress". Sixteen projects have achieved full-fledged Eureka status, with 25 more close on their heels.

But the last ministerial conference on Eureka held in Hannover set 31 January 1986 as the deadline for establishing the location and structure of the Eureka secretariat, and that deadline has passed with no decisions on location, although the group did agree on the basic structure and scope of the secretariat.

The site for the secretariat is significant for several reasons. While Brussels is favoured by a majority, some countries are concerned that Eureka should not become a satellite of the European Economic Community, especially since six of the

coordination of activities. One key decision of the recent meeting of Eureka High Representatives was to set approval of plans for the secretariat at the administrative level, rather than by formal treaty.

A provisional mechanism for approving projects for inclusion in the Eureka scheme is also now in place. Once two companies agree on a cooperative venture, there will be a 45-day period during which other countries may suggest their own companies as partners to the proposed project, although there is no guarantee that partnership status will in the end be granted.

Oakley sees opening up the market as a key role of Eureka. He is anxious to expand the international awareness of member companies, in part through management seminars bringing together corporate executives, financial experts and civil servants familiar with global issues.

Another important role for Eureka will be to encourage European standardization, perhaps along the lines of the British concept of "Eurotype", so as to smooth the path to market of Eureka projects.

According to Oakley, Eureka now enjoys full support from the British government. Whether it will ultimately succeed will depend not on the show of support it receives, but rather on the successful products it brings to the marketplace.

Joseph Palca

European neutrons

British aiming to share costs

THE British hope to gain substantially from attempts now being made to internationalize European neutron and synchrotron radiation sources. Hopes turn on a meeting arranged by the British Science and Engineering Research Council (SERC) for next Tuesday (18 February) at which representatives of France and Italy will discuss proposals for a substantial extension of the Spallation Neutron Source, commissioned a year ago at the Rutherford Appleton Laboratory in Oxfordshire.

The proposed extension of the Spallation Neutron Source, which goes under the new name of ISIS, would cost £60 million over ten years, of which a half would be met by SERC and the remainder by the other participants. But overseas participation in the project is a trade-off against British collaboration in the European Synchrotron Radiation Facility (ESRF) to be built at Grenoble in France, and to which the SERC contribution will be more than 15 per cent of the total construction cost of £150 million, at £24 million.

According to officials of potential partners, SERC has indicated unofficially but firmly that British participation in ESRF will be forthcoming only if the cost of the proposed ISIS project is shared with European partners. Indeed, SERC is looking not merely for a 50 per cent contribution to the cost of the extension, but for an "entry fee" of roughly a half of the cost of building the neutron source as it stands, which might amount to a total of £50 million. If these hopes materialize, SERC will be laying out £26 million less than it receives by way of contributions to ISIS.

Apart from France and Italy, other possible participants in the ISIS project include Denmark, Sweden, the Netherlands and Spain, some of whom may be

represented as observers at next week's meeting. West Germany will also be in attendance, but only as an observer; the West German plan to build a superior spallation neutron source, the SNQ, was cancelled only last year.

More recently, the intriguing prospect has arisen of Australian collaboration in the British neutron projects, although the potential European partners have not yet been told of this possibility. Australia is apparently short of neutrons, and the proposed deal with SERC would give Australia free access to ISIS as a *quid pro quo* for a reduction of British spending on the Anglo-Australian Telescope, recently recommended by a SERC review committee.

Enthusiasm for an improvement of the existing spallation neutron source stems from the good performance of the machine during its first year of operation and from the circumstance that it is a rapidly pulsed machine, producing neutron beams with very high intensity. French physicists, less keen on more neutrons than the Australians, say that the existing machine offers experimentalists few advantages over the reactor-based neutron source now working at the Institut Laue-Langevin at Grenoble, but that the proposed extension could be more interesting.

The status of next week's meeting is not yet crystal clear. The goal, at that and at a nominally separate but parallel series of meetings on ESRF, is to reach inter-governmental agreements on the two projects by the middle of next year, if all goes well. But French sources say that the French interest in next week's meeting and the subsequent negotiations is to discover "what part of ISIS can be Europeanized", which would seem to offer the British less than they are looking for.

Robert Walgate



18 nations participating in Eureka do not belong to the Community. But a more sticky objection to Brussels has been raised by the French. France has been the moving force behind Eureka since its inception, and Strasbourg is the French choice for the headquarters.

Brian Oakley, current chairman of the Eureka High Representatives, calls the delay in selecting a site "very annoying", but he believes that the situation may be resolved next March. By then the French elections will have passed, and it will politically acceptable for France to drop its insistence on Strasbourg.

The powers of the new secretariat are also important, as the smaller members are concerned to maintain some political muscle against the big four (France, Britain, West Germany and Italy). The new secretariat will consist of 12 people, half of them professional staff. Its principal function will be information dissemination and

British science

Sir Keith Joseph regrets . . .

It would have been "preferable" if the great cutback of British universities since 1981 could have "gone more gently", according to Sir Keith Joseph, Secretary of State for Education and Science for the past five years. Sir Keith, just now, is in a reflective mood. Ten days ago, he announced that he would not run for re-election to the House of Commons, where he has represented a Yorkshire constituency since 1956. But in an interview last week, he denied newspaper reports that he would leave his present post before the next general election, which must be held sometime in the next 27 months, saying that that is "entirely up to her", a reference to the Prime Minister, Mrs Margaret Thatcher.

Joseph is also unrepentant. Five years ago, he said, the British academic establishment was in need of "jolts". "We inherited massive overspending on research", while the universities were "snobbishly" disdainful of "trade". The result of the 8.5 per cent reduction of university budgets, Sir Keith insisted, was that some universities are now much better than they had been. He claimed some credit for having persuaded Sir Edward Parkes, chairman of the University Grants Committee when the budget reductions were enforced, to admit as much in public.

But have not other universities been damaged? Sir Keith acknowledges that academic morale may be at a low ebb, but insists that it is not yet appreciated in Britain that there is "no god-given right" to suppose that even the present level of prosperity in Britain can be sustained. The government is, however, worried about the loss of bright young scientist from Britain, and awaits with interest and anxiety the Royal Society report on the subject due in the early summer.

Sir Keith lists the causes of the emigration now under way as the invidious comparison between Britain and other countries in respect of salaries, equipment and the chance that good research proposals will be supported by the grant-making agencies, and says he thinks the last may be the most important. He did not last week include in his list the greater intellectual stimulation now to be found at universities outside Britain.

Sir Keith is known to be the chief monetarist influence on the present British government. Indeed, he is proud of having given 150 talks at universities in the two years before the 1979 election on "the moral case for capitalism", running into rowdy trouble on only six occasions. "D'you know", he says, "I went into politics because I wanted to get rid of poverty?"

Now, with the economic "chickens

coming home to roost" in Britain, "what we need are flocks and flocks of entrepreneurs". Sir Keith asks for agreement to that proposition (which is given) and to the proposition that the universities cannot produce them (which is withheld). There follows a scrappy conversation about the changing climate in British universities, and the reasons for the economic



success of the United States and Japan. Sir Keith seems to be advancing two contradictory propositions, that universities cannot, because of their constitution, be a prolific source of enterprise, but that, until they are, they will not be dealt with kindly.

On one specific point, the argument of the Committee of Vice-chancellors and Principals that the demand for student places seems more buoyant than the government's demographic projections, and will be further increased if Sir Keith's own plans for improvement in the schools succeed (see *Nature* 319, 348; 1986), the minister appears more willing to contemplate a re-expansion of British universities than previous statements would suggest. But he does not think that his plans for better schools will yield increased demand for higher education at least until the 1990s, which is probably a prudent calculation given the continuing conflict between the government and the teachers' labour unions.

On another point, Sir Keith admits failure. He points out that he has been publicly at odds with his colleagues in the government about the introduction of a scheme for supporting students at universities by means of loans. Such schemes work well elsewhere, he says; he still regrets the "honourable paternalism" and the fear that students from less prosperous backgrounds would be discouraged from seeking higher education, which appear to have been the chief reasons why the government backed away from student loans. Meanwhile, nothing seems to be happening to meet the vice-chancellors' com-

plaint about the impoverishment of students and their families.

That complaint is one of the many critical responses to the government's discussion paper on higher education, published a year ago. Sir Keith appears to be a little chastened by the reaction to the document. "If I got the tone wrong, that's my fault." But he resents having been called a "barbarian" on account of it.

So is there any way in which the universities in Britain might work their way back into the government's good books? The minister offers three tests — the abandonment of homogeneous salary scales for academics (which he says he has already brought up with university representatives), the abolition of tenure (and "you know what we are doing about that") and the pursuit of rigour in the universities (to which end the universities' current examination of academic standards will contribute).

Much of what applies to the universities applies to Sir Keith's views of publicly supported research in Britain. Again, it would have been preferable that the "financial adjustments" required of the research councils had been spread over, say, a five-year period. Again, it would help if there were better links, both ways, with the outside world and with industry in particular. There is a case for a science minister, but not an overwhelming (or, one gathers, a persuasive) case. There is no reason to think, he says, that matters such as the British membership of the European high-energy physics community (CERN), or the question of the privatization of the Plant Breeding Institute, would have been settled any more quickly if there had been a minister responsible for getting things done.

In private, it is known that Sir Keith Joseph has in recent years been fighting hard to preserve the publicly supported research base in Britain. Aloud, he remarks on the anomaly that, when his post-bag was full of letters from parents protesting at his scheme for raiding the budget for student support in the interests of research (at the end of 1984), he found only one letter of thanks from a scientist. Looking back on his time in his present office, he says with pride that he is glad to have identified a number of problems of national importance (to the British); plainly, he also regrets not having done more to solve them.

So what can the research community in Britain do to win back the support of the government? No doubt a strong show of entrepreneurship would go a long way to meet Sir Keith's demands. But he himself offers an unexpected suggestion; why not win friends in the House of Commons? Perhaps he is feeling more lonely there now than when the government to which he belongs came to office six years ago.

John Maddox

Ban on South Africans

SIR—Your first leading article on the question of the South African ban (*Nature* 318, 195; 1985) castigates the executive committee of the World Archaeological Congress for bowing precipitately to pressure from non-academic bodies. Your second (319, 85; 1986) fails to mention that a meeting of the full national committee, which includes most of the scholars organizing the individual academic sessions of the congress, convened on 20 November, supported the ban by a considerable majority and reiterated that this was done on grounds of principle rather than pragmatism.

It is, however, clear from the tone and content of both articles that the committee's affirmation of the ban would have had little effect on your general arguments, which could be summarized as stating that while there may be — grudgingly — a case to be made for economic or sports sanctions, there is no case for academic sanctions. The notion that "academic freedom" is an unproblematic concept, that academics have different and more elevated concerns than other people, that academic debates can be, indeed should be, divorced from historical, human and political reality requires vigorous refutation.

This archaeological congress has from the outset laid particular emphasis on being a *world* congress. Contributions from countries outside the industrialized West were particularly solicited, funds were found to make it possible for people to come to England, and themes were selected that did not simply reflect the preoccupations of "the North". The South African ban must be seen as part of this vision of what an international congress should be, and it must be recognized that failure to implement the ban would lead to the withdrawal of many participants, both from other parts of Africa and from countries expressing solidarity.

Participants at the world congress register as individuals, but their national affiliation is published. The question of admission of South Africa to an international forum cannot therefore be avoided. It might be argued that exclusion isolates and devalues the work of individual academics who have fought against apartheid from within the system. But the ban is not about individuals, it is not, indeed, about academics, it is about bringing pressure to bear on the South African government by boycotting interchanges of any sort, and this policy has the complete support of those organizations within South Africa that speak for the majority of that country's people.

It might be argued that banning South Africa is hypocritical — if South Africa, why not all the other countries that prac-

tise violent repression or genocide of "undesirable" ethnic groups? There are two reasons. First, that, unlike the other regimes, in South Africa ethnic stratification is institutionalized as the basis for formal exclusion of the majority from political rights. Second, perhaps more important, there are "historical moments" when particularly repressive action by a regime and reaction by the oppressed reach a point where positive "indignation" expressed in *every* form available to the outside world may have some effect. The state of emergency declared by the South African regime is one such moment.

One of the aims of academic research in the social sciences is to expose the realities of human social life, and to analyse the bases and causalities of what we observe, but if we restrict our discussion to academic forums, if we deem ourselves above politics, we draw the teeth of "academic freedom" and make it into something esoteric and safe. Academics cannot insulate themselves from politics. We have been asked by those within South Africa who risk their lives in opposing the regime to help them by imposing a ban. If we do so, we may also bring pressure to bear on Western governments by convincing them of the strength of their own public opinion. If, in the name of academic freedom we do nothing, we are, whether we like it or not, making a political statement — a negative one.

Your editorial called for plain speaking, and persuasion in the interests of tolerance. We all agree on the need for plain speaking. What some of us are left wondering is what it is you are asking us to tolerate.

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Star colours

SIR—A recent paper¹ suggests that some Babylonian, Graeco-Roman and early-medieval authors considered Sirius to be red in colour. Today it shines white. I wish to argue here that, according to ancient Chinese evidence, (i) Sirius had been white all along but (ii) Betelgeuse has changed colour from yellow to red.

Volume 27, *Chronicles of Celestial Officials*, in *Historical Records* edited by Sima Qian of the Han dynasty, in the first century BC, contains the following passage: "The white is like Sirius, the red like Antares, the yellow like Betelgeuse, the blue like Bellatrix, and the dim like

Mirach".

To my knowledge the above assessment was not challenged in any of the twenty-three dynastic histories compiled since, which all include chapters based on materials supplied by astronomers royal.

It is possible that the purported attribution of a red colour to Sirius in Babylonian cuneiform texts is a case of mistaken identity². Assertions to the same effect in classical works may be secondary citations by non-expert authors.

In contrast to Sirius, Betelgeuse (α Orionis) does appear to have altered its colour in the light of ancient Chinese sources. It is now red, with $B-V=1.9$, comparable to the index for Antares (1.8) which the Chinese did regard as red. Being massive, however, Betelgeuse could well have cooled from yellow to red within 2,000 years, during the end of its evolution away from the main sequence into a red supergiant. For Sirius to leave the red giant and reach a white dwarf stage in a similar span of time would be an anomaly, if we accept the current models of stellar evolution¹.

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Radiation threshold

SIR—John Simpson's review of four books on British nuclear testing in Australia (*Nature* 318, 319; 1985) claims that the "official view" about the relationship between radiation exposure and ill health and premature death is "that threshold values can be established to distinguish between those who are 'safe' and those 'at risk'". This is incorrect. The concept of a threshold was in common use during the 1920s and 1930s. For at least the past 35 years, however, radiological protection has been based on the assumption that there is no threshold below which it can be assumed that radiation has no harmful effects. When the International Commission on Radiological Protection was reformed after the war, it made recommendations of maximum permissible exposures at a level that involved a risk small compared with other hazards of life. Subsequent developments of radiological protection philosophy and practice have all been based on the linear no-threshold hypothesis, and it seems increasingly likely that, at least for some important types of radiation, this hypothesis results in an overestimation of the risks¹.

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- Report of the National Research Council Committee on the Biological Effects of Ionising Radiation (BEIR), National Academy Press, Washington, DC, 1980.

More tests of special relativity

Yet another experiment has provided a novel test of special relativity, one that duplicates in the laboratory phenomena for which people usually look to astrophysics.

GIVEN the zeal with which those who disbelieve the special theory of relativity go about their business, it is a comfort that experimental tests of relativity are also flourishing. One of the latest, an experiment carried out at Los Alamos by D.W. MacArthur *et al.*, has the special virtue of providing a test, successful as it happens, of the relativistic Doppler effect at velocities as great as 0.84 of that of light (see *Phys. Rev. Lett.* **56**, 282; 1986). Moreover, this laboratory experiment has all the promise of being generically novel.

The principle of the experiment is straightforward enough, as most convincing designs must be. The essential phenomenon is the excitation of quickly moving neutral hydrogen atoms by an ultraviolet laser. The atoms of the hydrogen beam have essentially a constant velocity, as does the laser. But by varying the angle at which the laser beam intercepts the moving hydrogen atoms, the interaction energy — as seen from a frame of reference in which the atoms are at rest — can be varied over a wide range.

The experiment has been made possible by the meson factory at Los Alamos, formally known as the Clinton P. Anderson Meson Physics Facility (LAMPF) and, as such, an enduring memorial of the time when members of the US Congress were among the strongest supporters of the nuclear industry in the United States. Among other things, the machine can produce a beam of negative hydrogen ions with an energy of 800 MeV, corresponding to 0.84 of the velocity of light.

Magnet

The beam is processed in three successive steps. First, a proportion of the negative hydrogen ions is converted into neutral atoms by means of an infrared laser at a frequency corresponding to that needed to shake loose one of the two electrons. The second and crucial step is the interaction with the beam of a pulsed ultraviolet laser, an yttrium aluminium garnet device doped with neodymium and yielding photons with an energy of 4.66 eV. The effect of the interaction is, naturally, to excite some of the neutral hydrogen atoms or even to ionize them completely. The final step is the separation of the three species of hydrogen atoms, the neutrals and the negatively and positively ionized atoms, by a magnet. The trick is to use a magnet whose field is strong enough to convert into protons any neutral hydro-

gens that have been excited above the second excited state (with principal quantum number three). Both the neutral hydrogens and the protons are counted by means of scintillators.

The cumbersome part of the equipment is the turntable that allows the laser beam to intercept the atomic beam at various angles. (There is a system of mirrors to ensure that the laser itself does not move.) The result is that the energy of the ultraviolet photons appears, from the rest-frame of the hydrogen beam, to vary by an order of magnitude between 1.4 eV and 15.8 eV. For practical purposes, the arrangement is one for allowing the measurement of the energy of the successive excitations of neutral hydrogen atoms. The authors of the experiment show convincingly that the excitation curves that they obtain (essentially the production of protons in the detection system as a function of the angle of their turntable) are indistinguishable from a gaussian curve (which is what would be expected). They have been able to record resonances corresponding to excitations from the ground state to between the fourth and the eleventh quantum level inclusive. The interpretation of the data hangs on the accuracy with which the position of the turntable can be measured, with a precision of 30 microradians, it is claimed.

Reconciling these results with what special relativity predicts requires a little subtlety. The familiar Lorentz transformation between one coordinate frame and another has the time T in one frame of reference related to that, t , in the other by means of a linear relationship of the form $T = at + bx$, where x is a spatial coordinate and the factors a and b are coefficients depending on the relative velocity of the two frames, v . In practice, the prediction of special relativity is that the quantity a is the time-dilation factor given by the square root of $1/(1 - v^2/c^2)$. MacArthur and his colleagues allow the possibility that this relationship may not be exact, that the coefficient a may be a function of velocity other than the time-dilation factor, and set out to determine from their resonance measurements just what it may be. Because of the way in which the measurements are carried out, by the interaction of atoms and photons at positions that are well-defined in both frames, the experiment cannot provide information bearing on the second coefficient, b .

Unsurprisingly, the outcome of the experiment is that the time-dilation factor is sufficient to account for the variation of the energy of the ultraviolet photons as seen from the frame in which the hydrogen atoms are at rest. That energy is the energy of the photons in the rest-frame of the laser multiplied by the time-dilation factor and by the factor that represents the influence of the strictly classical Doppler effect. This, by involving the cosine of the angle between the two beams, allows for the way in which the experiment spans a whole order of magnitude in the excitation energy of the neutral hydrogen atoms. The precision of the result is given as 27 parts in 100,000. With what would obviously be a great deal of hard work, the authors believe they could improve the precision of the experiment by a factor of as much as 1,000.

Velocity

The arresting feature of the experiment now described is not, however, its precision but the large fraction of the velocity of light at which the two frames are moving relatively to each other. It is true, of course, that most people are sufficiently persuaded of the accuracy of what has now been demonstrated about the accuracy of the time dilation factor of special relativity by the increase of the lifetime of quickly moving particles, as in cosmic rays, while the behaviour of charged particles in accelerating machines is another line of related evidence. Another novel feature of the Los Alamos experiment is that it provides a demonstration of how a laser beam, with a frequency defined in one frame of reference, can be translated into a tunable but defined frequency in another. That should be suggestive of all kinds of other measurements.

In the long run, efforts in that direction may be less rewarding than would be a successful experiment to verify that the other factors in the Lorentz transformation of coordinates behave as predicted. The obvious difficulty is that of constructing experiments in which events seen as if from two relatively moving frames are separated from each other in time and/or distance. The obvious measurements to make are those of the signals from Earth satellites and spacecraft. Much has been done already, but further efforts are needed, and not merely to keep the army of sceptics of the special theory at bay.

John Maddox

Biochemistry

Polyribonucleic acids as enzymes

from F.H. Westheimer

In the January 31 issue of *Science*, A.J. Zaugg and Thomas Cech¹ published a paper entitled *The Intervening Sequence of Tetrahymena is an Enzyme*. This publication seems destined to take its place among the classics in biochemistry.

Sixty years ago, James Sumner crystallized urease² and, over the vigorous objections of Richard Willstaetter, set about proving that his crystalline protein was in fact the enzyme responsible for the hydrolysis of urea. In subsequent years, many enzymes have been purified, and the scientific community accepted — or more precisely had accepted — that all enzymes are proteins, where enzymes are defined as biological catalysts that accelerate metabolic processes by factors of 10^6 – 10^{12} . In keeping with the knowledge available Lubert Stryer³, in his excellent text *Biochemistry*, states flatly that the biological catalysts "are specific proteins called enzymes". Now Cech and his co-workers at Colorado (and in complementary work, Sidney Altman and his co-workers at Yale) have established that polyribonucleotides can function as enzymes. The discovery is revolutionary in its direct impact on the theory of catalysis, in its promise for future discovery and in its implications with respect to the pathway of prebiotic chemistry. Additionally, it carries with it a series of challenges for understanding the chemistry of phosphates.

Discovery

First, a few words about the discovery itself. An RNA from the protozoan *Tetrahymena thermophila* contains, in addition to the RNA of the ribosomes, an intervening sequence (IVS) of 413 nucleotides: the mature ribosomal RNA is formed from this precursor by the precise removal of the intervening sequence at its junctions with the ribosomal sequence, and the splicing of the two ends (see Fig. 1). In 1981, Cech and his co-workers showed, astonishingly, that the excision of the intervening sequence does not require an enzyme — at least not in the conventional sense — but was initiated by the addition of micromolar quantities of GTP, or (as was later discovered) by almost any derivative of guanosine, including guanosine itself. The nucleoside initiates a series of moderately rapid reactions that lead to the excision of the intervening sequence without the need for a protein catalyst^{4–7}.

The guanosine (or guanosine derivative) attacks the RNA at the 5' end of the intervening sequence of 413 nucleotides; the reaction is one of ester exchange, where the 3' hydroxyl group of the guano-

sine attacks the phosphodiester bond between the 3' end of the first exon and the first nucleotide of the intron. In the resulting product, the guanosine residue is attached to the 5' end of the intron. Subsequently the 3' end of the 'liberated' exon spontaneously attacks the far end of the 413-base intron to splice the two ends of the exon together (Fig. 1). Cech comments that these transesterification reactions are thermodynamically neutral, and so require no GTP or ATP to drive them forward. True, the transesterifications are essentially neutral with respect to the enthalpy of reaction, but to the extent that they go forward nearly to completion rather than coming to balanced equilibria, there must be a driving force for the overall process. In some instances, for example where a small molecule is ejected, the reactions are entropy driven; in others, the driving force must be related, at least in part, to changes in stability between reactants and products that arise from changes in the tertiary structures of the RNAs themselves.

After the intron has been extruded and the exons spliced together, the free intron undergoes a cascade of spontaneous reactions (Fig. 2). First, the 3' end of the poly-

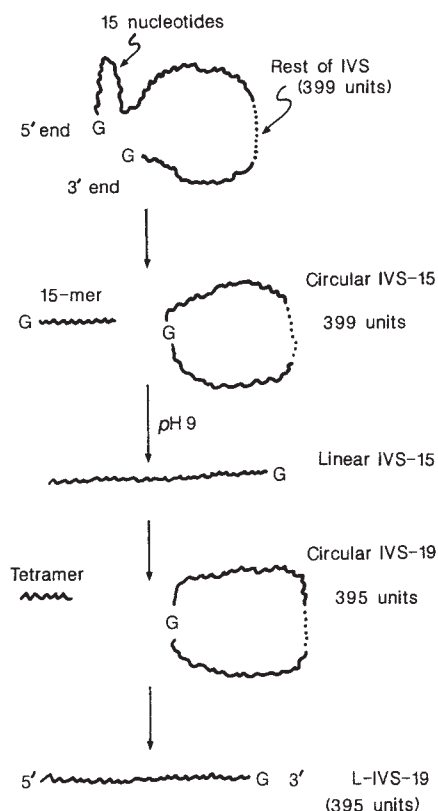


Fig. 1. Highly schematic diagram of self-splicing in the RNA of *Tetrahymena*.

ribonucleotide attacks, nearly specifically, the phosphate between the 15th and 16th nucleotides (from the 5' end) and forms a circular polynucleotide; the process results in ejecting 15 nucleotides from the 5' end of the intron. If the resulting circular polynucleotide is then exposed to higher pH (9.0 instead of 7.5), the ring is opened at precisely the same bond where it had been formed; then the active 3' end bites again, now four nucleotides from the 5' end, to eject a tetramer and yield a new circular polynucleotide. This ring in turn is opened by the mildly alkaline solution at precisely the bond where it closed to yield a polyribonucleotide that is 395 units long. Here the cascade of reactions stops; but it is this truncated linear intervening sequence (L-IVS-19) that has enzymic properties that are the subjects of Zaugg's and Cech's paper in *Science*.

Specificity

The spontaneous reactions producing the intervening sequence are remarkable enough. They are fast and quite specific, and carry strong implications of specific tertiary structures and specific binding sites in RNA. One is tempted to say that one part of the molecule is catalysing a reaction at another site within itself. But technically the reactions are not catalysis; by definition, a catalyst accelerates a reaction without undergoing change itself, or else is regenerated unchanged at the end of the process.

Many organic molecules undergo rapid internal reaction that is not catalysis. A spectacular example⁸ concerns the cyclization of 2-hydroxy-phenylpropionic acids to dihydrocoumarins. A pentamethyl derivative of the acid undergoes acid-catalysed closure some 10^{10} times as fast as the unsubstituted compound does; the lactonization occurs at a rate that could be achieved by phenylpropionic acid in phenol at a hypothetical concentration of 10^{15} M. Presumably the methyl groups cause strain that is relieved on lactone formation. No one is about to call these phenolic acids enzymes, even though their rapid reactions are noteworthy. The internal reactions that Cech discovered are, of course, much more remarkable, largely because of their extraordinary specificity, and because of the distance between the reacting groups. The aromatic acids cited above have only one place to react; although the RNAs have thousands, they react at precise loci. Nevertheless a purist can maintain that, although the molecules are activated for internal reactions, they are not true catalysts. That argument — or quibble, if you prefer — does not apply to the piece of intervening sequence, L-IVS-19, that results at the end of Cech's cascade. It is a true enzyme.

In the presence of the truncated intervening sequence, various oligoribonucleo-

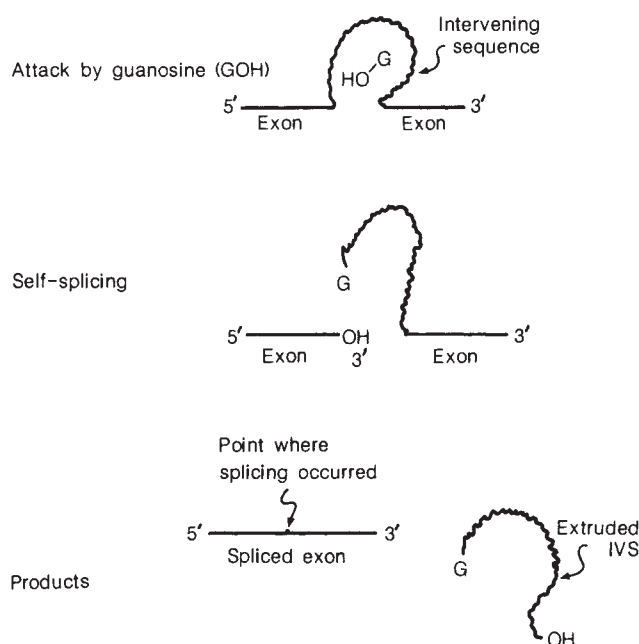


Fig. 2. The cascade of spontaneous reactions of the IVS.

tides undergo hydrolysis at rates of about 2 per minute. That is not extraordinarily rapid, but it is fast compared with the spontaneous rate of hydrolysis of RNA, and corresponds roughly to the rate of hydrolysis of DNA as catalysed by restriction enzymes, or to the rate of reaction catalysed by staphylococcal nuclease, or by a nonspecific phosphodiesterase⁹⁻¹³. Of course, RNA is generally much more liable to hydrolysis, both with alkali and with enzymes, than is DNA; pancreatic ribonuclease has a turnover number of thousands per second. But both pancreatic ribonuclease and alkali are able to use the adjacent 2' hydroxyl group of a ribose residue to cleave the phosphate ester bond of the RNA. It is this internal attack by an adjacent residue that accounts for the rapidity of the reaction; and it is of course the absence of the 2' hydroxyl group that makes DNA more stable to hydrolysis. The reason that Cech's IVS hydrolyses RNA relatively slowly is apparently that it fails to exploit the 2' hydroxyl group: this is inferred from the fact that Cech's IVS yields a free 3' hydroxyl group and leaves a phosphate at the 5' position, whereas pancreatic ribonuclease leaves the phosphate at the 3' position and yields a 5' hydroxyl group as the almost inevitable consequence of the internal reaction with the 2' hydroxyl group. This poses an interesting problem for the mechanism of catalysis.

In a series of studies with pentacytidylic acid (C_5), Zaug and Cech have now shown¹ that the truncated intervening sequence acts on this oligonucleotide to yield a mixture of polycytidylic acids, including C_3 and C_4 , together with longer molecules up to C_{30} . Here again, the transesterification reactions are probably essentially thermoneutral; here the reac-

tion nucleotides of *Escherichia coli* to create transfer RNAs, and in particular to cleave a precursor to fashion the correct 5' terminal ends for these molecules. Ribonuclease-P, like Cech's IVS, cleaves ribonucleic acids the 'wrong' way, that is, it cleaves a 3'-5'-phosphodiester bond to leave the phosphate residue on the 5'-rather than on the 3'-position. Altman and his co-workers showed that ribonuclease-P contains ribonucleic acid as well as protein, and then discovered that the enzymic activity, although it is promoted by protein, resides in the nucleic acid component¹⁵. This discovery was sandwiched between Cech's finding of self-splicing in *Tetrahymena* RNA and that of the catalytic properties of the IVS.

Ribosomes

Subsequent to these two seminal discoveries — that of Cech and that of Altman — several other investigators^{16,17} have found more self-splicing RNAs, following the model that Cech introduced. And other enzymes^{18,19} contain both ribonucleic acid and protein, although it is not yet known whether the nucleic acid portion has catalytic properties. Furthermore, these new discoveries once again focus attention on ribosomes, where the complicated interrelationship of protein and ribonucleic acid must still be untangled^{20,21}. Just when chemists were beginning to think that they could account, at least in part, for the catalytic properties of enzymes — protein enzymes, that is — in terms of structure and mechanistic principles, we are faced with new, unexpected and exciting problems. What are the tertiary structures of the active ribonucleic acids? Presumably their tertiary structures (and those of their substrates) are vitally important; without tertiary structure, the specificity of the

tions are presumably entropy driven, yielding a complex mixture from a single starting material. There can be no doubt that the IVS is acting as a true catalyst, and must be considered an enzyme.

The result is startling, but even now is not unique. First and foremost, in entirely independent but complementary research, Sidney Altman and his collaborators have also demonstrated the catalytic (the enzymic) action of particular polyribonucleic acids. They had, for many years, studied ribonuclease-P¹⁴, an enzyme that cleaves

catalytic polyribonucleotides would be hard to understand. What are the active sites of these new enzymes? What principles explain their activity? Can Cech's IVS, or Altman's catalyst, be crystallized? The IVS has about 400 residues. In a sense, this is comparable in complexity to a protein enzyme. Characteristically, proteins have 300–400 residues, and if the molecular mass of each residue is only about a third as large as that of nucleotides, the inherent diversity of the amino acids is much greater.

The new discoveries bring with them speculations about prebiotic chemistry²². Bass and Cech²³ have wondered whether the ribozymes may not have preceded protein enzymes. As ribonucleotides apparently possess both the properties of self-replication and those of catalysis, could these molecules have allowed a primitive living system to function without either DNA or protein? The observation that ribonuclease-P and other enzymes contain both nucleic acids and protein suggests a pathway for prebiotic evolution. Polyribonucleic acids could have accumulated amino acids and short peptides by hydrogen-bonding them, provided that these protoproteins helped the polyribonucleic acids with respect to stability or catalysis, or both. Over time, the protein component of such mixed enzymes could have gained in complexity, and eventually the greater versatility of the amino-acid residues, compared with those of the nucleic acids, could have allowed the protein component to take over; in this scenario, in most instances the enzymes then sloughed off the nucleic acid part. Could enzymes such as ribonuclease-P be biochemical fossils, that help to illuminate for us the pathway of biochemical evolution?

But putting such speculation aside, the chemistry pioneered by Cech and Altman and their co-workers is already leading to much new biochemistry. On the one hand, several investigators are finding cascades of reactions similar to that that Cech discovered with *Tetrahymena*. On the other, the mechanistic problems in the cleavage of phosphodiester bonds and especially those problems that arise because of the cleavage of the 3',5'-diester bond to leave the phosphate residue on the 5' position, stand as challenges to mechanistic biochemists. □

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100 years ago

Mr Johnston was early in 1884 specially commissioned by the Royal Society and the British Association to study the interesting fauna and flora of the Kilima-Njaro uplands. During the six months from May to October of that year, this experienced African traveller has succeeded under great difficulty in collecting abundant materials for illustrating the natural history and physical constitution of the "Mountain of the Snow Fiend," as its euphonious Ki-Swahili name is interpreted. These results are embodied in the work before us, which is alike admirable for its bright and graphic style, and the judicious arrangement of its varied contents. By the simple plan, consistently adhered to throughout, of treating the narrative portion separately, and confining the strictly scientific matter to the concluding chapters, all tastes are consulted, and the common mistake is avoided of sacrificing the interests of the student to those of the general reader: "The Kilima-Njaro Expedition, a Record of Scientific Exploration in Eastern Equatorial Africa." By H.H. Johnston, F.Z.S. (London: Kegan Paul, 1886).



Amongst the valuable animal specimens secured was one of the new and beautiful species of Colobus (*C. guereza*, Rüpp., var. *caudatus*, var. nov.) first seen and described by Mr. Thomson, which frequents the base of Kilima-Njaro, and is apparently restricted to that region. Mr. Oldfield Thomas tells us that it is "characterised by having the white brush of the tail very much larger and finer than is the case in the true Abyssinian *C. guereza*..." From *Nature* 33 322, 4 February 1886.

Biological rhythms

Sleep researchers caught napping

from N. Mrosovsky

MINIMA in body-temperature rhythms usually occur towards the end of each night's sleep. When people live in constant conditions, without information about the time of day, sleep and temperature rhythms can become desynchronized. Initially, both rhythms free-run together with a circadian period of about 25 hours and then, in some subjects, the period of the sleep rhythm suddenly lengthens to a value near to 30 hours while the temperature rhythm retains its 25-hour period. Up until now, this has been the best example of 'internal desynchronization'; it has been used as evidence that circadian rhythmicity depends on several biological clocks, as one oscillator cannot run at two different frequencies simultaneously. The data on which the argument rests have recently been re-analysed by J. Zulley and S.S. Campbell (*Human Neurobiol.* **4**, 123; 1985 and *Sleep* **84** (eds Koella, W.P., Rütter, E. & Schultz, H.) 81; Fischer, Stuttgart, 1985).

The question of whether there are one or several oscillators controlling circadian rhythms is not only of great importance to specialists on rhythms, but also has practical implications in understanding adjustment to shift work. It is also an essential ingredient of some recent theories about the role of biological rhythms in depression and cycles of mania and depression.

Both presentation and selection of data have contributed to the view that sleep and temperature can become desynchronized. Rhythm data are frequently double-plotted — a given time-segment, say 24 hours, appears twice, once below the previous segment and once beside it (on the right in Fig. 1.) This makes it easy for the viewer to see continuity in data spanning two 24-hour periods, rather than having to glance back to the start of the 24-hour segment below. The principle of double mounting is often extended to treble and multiple mounting.

Figure 2a shows an extended plot of data on major sleep episodes using the multiple-mounting method. In this case, however, the principle of showing continuity has been carried even further by plotting each sleep episode only once. This extended plot draws the eye across the page in a way that emphasizes differences in the periodicity of the sleep and temperature rhythms. Textbook illustrations are often in a format similar to that of Fig. 2a. When the sleep episodes are also plotted below each other (Fig. 2b), it is much less clear that the periodicity of sleep and temperature are different. But there do remain some days when sleep is

apparently absent at the times of temperature minima, which is evidence for a long periodicity in the sleep rhythm.

But what is sleep? Here we come to the problem of what to include in the database. In classic diagrams showing internal desynchronization, only times that the subjects themselves considered to be major sleep episodes were included and naps were excluded. In fact, subjects were instructed not to take naps but to remain active between the times that they experienced as night. Some subjects were either unable or unwilling to follow these instructions. If they decided to sleep outside what they considered to be their major bout of night-time sleep, they pressed a button indicating they were taking a nap. But, as Zulley and Campbell point out, some of these 'naps' lasted several hours, as long as major sleep episodes. When such naps are included in the plots (see Fig. 2c), they cluster around the time of the temperature minima. There are also some shorter naps in the middle parts of what is perceived as day, but whatever it is called, some sleeping takes place nearly every day around the time of the temperature minima. The evidence for internal desynchronization disappears — almost.

To salvage the hypothesis, it has to be postulated that internal desynchronization occurs between what the subject per-

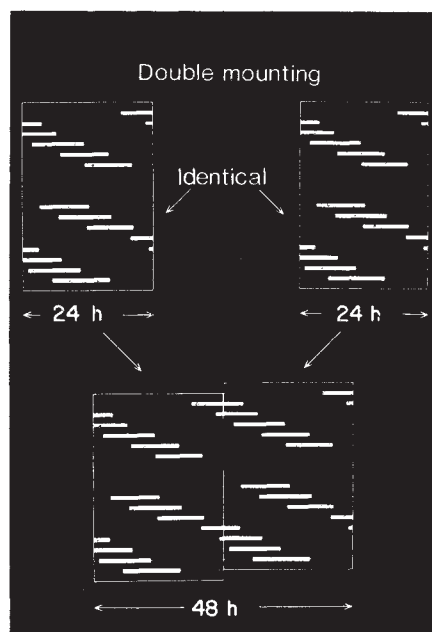


Fig. 1 Double-plotting method for showing data on biological rhythms. Two identical photographs of the same data are mounted side by side to give consecutive 24-hour spans across, as well as down, the page.

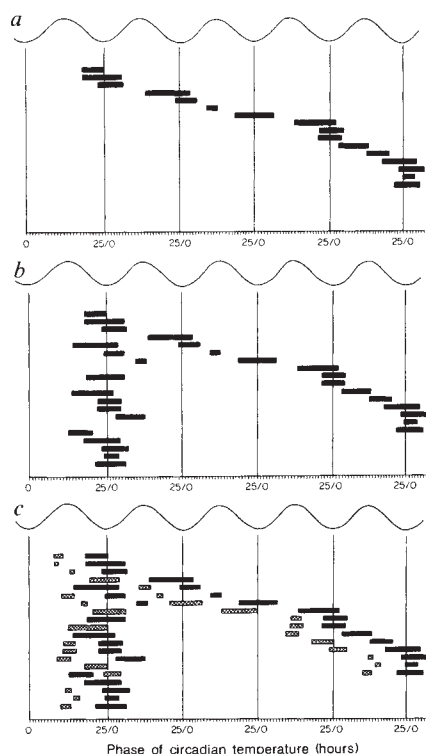


Fig. 2a, b, c. The body-temperature rhythm of a person in constant conditions free running at a period of 25 hours is shown schematically (line and 25-h scale on abscissa). The subjectively perceived major sleep episodes (black bars) are shown in extended plot only. **b.** The same data as **a** but with the sleep episodes plotted twice. **c.** The black bars are the same major sleep episodes as shown in **a** and **b**. The hatched bars show sleep that the subjects designated as naps. Note that some sleep occurs nearly every day around the time of temperature minima. Curved lines in **a-c**, body temperature (schematic). (Redrawn from Zulley and Campbell.)

ceives as the time for napping and the time for sleeping; in other words, what is perceived as day or night. Before this interpretation can be accepted, it is necessary to study the exact instructions given and the way they are interpreted. Zulley and Campbell necessarily selected subjects who napped despite instructions — perhaps internal desynchronization occurred in the other subjects, those that did not nap. To answer this it will be necessary to learn whether such subjects did not always feel sleepy when their body temperatures were low, or whether the instructions not to nap sometimes overrode sleepiness at those times.

A happy feature of Zulley and Campbell's work is that they have evidently been assisted, rather than resisted, by Jurgen Aschoff, one of the chief proponents of the phenomenon of internal desynchronization, who has made his data available and continues to exert creative leadership in the field. □

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Neurochemistry

Peptides and Alzheimer's disease

from Alan Fine

THE observation ten years ago that cholinergic enzyme activities are reduced in the brains of patients with Alzheimer's disease¹⁻³ triggered a surge of interest in the biology of acetylcholine in the mammalian forebrain. Since then it has become clear that the neurochemical pathology of Alzheimer's disease, although selective, is not restricted to cholinergic pathways. New discoveries of profound decreases in cortical levels of corticotropin-releasing factor in Alzheimer's brains, reported on page 593 of this issue⁴ and elsewhere⁵, reinforce this conclusion. What are the implications for the pathology of Alzheimer's disease?

Corticotropin-releasing factor (CRF), a 41-amino-acid-long peptide first characterized by W.W. Vale *et al.*⁶, and known to be a major hypothalamic regulator of secretion of β -endorphin and adrenocorticotrophic hormone from the anterior pituitary, has more recently been found in other neurones including those of the cerebral cortex. CRF-containing neurones are present in the neocortex of rodents, with cell bodies predominantly in laminae II and III and terminal fields mainly in laminae I and IV, where CRF receptors are also concentrated⁷⁻⁹. CRF is also contained in cholinergic neurones in the brainstem of rats, some of which may project to the forebrain, including the cortex.

What E.B. De Souza *et al.*⁴ and G. Bissette *et al.*⁵ now observe is a significant reduction in CRF (measured by radioimmunoassay) in several brain areas from Alzheimer's patients compared with matched controls. Both groups report greater than 50 per cent reductions in frontal and temporal cortex. The greatest reductions were found in occipital cortex (80 per cent) and caudate nucleus (70 per cent), at variance with the pattern of loss of cholinergic markers. Similarly surprising is that there is no significant change in CRF levels in the hippocampus, cingulate cortex, amygdala or substantia innominata, all regions where structural and cholinergic changes occur in Alzheimer's patients.

Previously, somatostatin was the only neuropeptide whose brain levels were known to be reduced in Alzheimer's disease^{10,11}. Two neuropeptides other than CRF, vasoactive intestinal polypeptide and cholecystokinin, are known to have high cortical concentrations (relative to subcortical levels) in the normal brain. They are not reduced in Alzheimer's disease, which indicates a biochemical specificity in the pathology of the disease.

De Souza *et al.* have also described a

proportional increase in CRF receptor levels in those areas with reduced levels of CRF. Thus, they find a twofold increase in CRF binding in the occipital cortex of Alzheimer's patients compared with controls. This up-regulation contrasts with the decreased somatostatin binding found in Alzheimer's brains¹².

It will be of interest to learn whether the distribution of CRF-containing neurones in the human brain is similar to that in rodents, and, if so, whether the observed CRF deficits reflect abnormalities of intrinsic cortical cells or of neurones that project to the brainstem. Furthermore it will be important to determine whether CRF neurones are associated with the neuritic (senile) plaques and neurofibrillary tangles that are the identifying histological abnormalities of Alzheimer's disease. Cortical somatostatin-containing neurones in Alzheimer's brains have been shown to contain tangles¹³, which has led to the supposition that they are a primary focus for the disease.

There is already evidence that CRF can modulate cholinergic behavioural effects and cortical somatostatin release in rats. More extensive studies of the physiology and behavioural pharmacology of these cortical neuropeptides are now needed. Intervention in any single neurotransmitter pathway thus seems to be unlikely to provide satisfactory therapy for Alzheimer's patients. Effective therapy may need to have multiple targets, with selective M_1 -agonist and M_2 -antagonist cholinergic agents (the latter to maximize the efficacy of remaining cholinergic terminals) used in conjunction with appropriate peptidergic or α -adrenergic¹⁴ agonists.

It has previously been suggested that degeneration of subcortical cholinergic (and perhaps noradrenergic and serotonergic) projection neurones of the so-called isodendritic core was the primary lesion of Alzheimer's disease^{15,16}. In this hypothesis, neurofibrillary tangles, neuritic plaques and neuronal loss in target areas of hippocampus, entorhinal cortex and neocortex represent the trans-synaptic consequences of this subcortical degeneration. However, as noted above, regional CRF changes are not proportional to cholinergic changes, nor does the cortical distribution of plaques accurately mirror the regional distribution of ascending cholinergic inputs (but plaques do parallel the distribution of somatostatin terminals).

Although cholinergic neurites have been identified in association with plaques in human¹⁷ and aged monkey brains¹⁸, so

also have somatostatin^{19,20}, γ -aminobutyric acid²¹ and catecholamine-containing neurites²². It is probable that cholinergic deafferentation of the cortex (including the hippocampus) is an important contributor to the cognitive impairments of Alzheimer's disease. However, the hypothesis that subcortical cholinergic neurones, by virtue of their particular metabolism or other molecular specialization, are the primary focus of Alzheimer's pathology seems no longer tenable. If cholinergic subcortical degeneration is not the primary focus, it may reflect a disease process acting concurrently on diverse independent structures, or it may be the result of abnormalities of the target cortical regions. The latter possibility is compatible with observations that the neuronal loss in different parts of the cholinergic basal forebrain system in Alzheimer's disease is proportional to the extent of neuritic plaque formation in their respective cortical targets²³, and with the observation of degenerative changes in cholinergic neurones of the basal forebrain following

cortical lesions in rats^{24,25}, monkeys and humans²⁶. □

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Atmospheric chemistry

Reservoir species discovered in the stratosphere

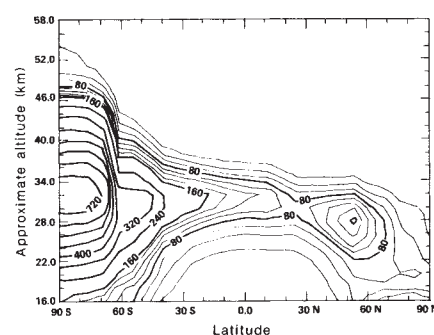
from Donald J. Wuebbles

It is now recognized from theoretical models that several trace constituents of the stratosphere affect the distribution of ozone. But because of difficulties in achieving enough sensitivity, the stratospheric concentrations of many of these species, particularly those termed 'reservoir' species, have remained unobserved. Now, Toon *et al.*, on page 570 of this issue¹, report the first conclusive measurement of the reservoir species N_2O_5 .

Reservoir species are those trace constituents, such as HNO_3 , HCl , N_2O_5 and ClONO_2 , that are produced from the more reactive ozone-destroying species, but are longer-lived and less reactive. In a sense, these gases act as temporary reservoirs or holding tanks for the more reactive ozone-destroying forms of nitrogen-, chlorine- and hydrogen-containing species in the stratosphere. Of these reservoir species, only HNO_3 and HCl have been measured extensively. The concentration distribution observed by Toon *et al.* applies only to a particular latitude and longitude on a single day, but nonetheless represents an important step towards the validation of key aspects in the theory of ozone-controlling mechanisms.

Many recent studies have concerned the possibility that increasing concentrations of trace-gas emissions caused by human use, such as methane, nitrous oxide (N_2O) and chlorocarbons (particularly CFCl_3 ,

and CF_2Cl_2) affect the distribution of stratospheric ozone. These trace gases, although emitted at the Earth's surface, are sufficiently long-lived to be transported to the upper atmosphere, where they dissociate into by-products that can



Two-dimensional model simulation of the latitudinal variation in N_2O_5 concentrations determined for May at local noon. By definition, the mixing ratio is the concentration divided by the air density. From the model of S. Solomon and R. Garcia.

react with ozone catalytically. The rate of conversion of the reactive species to the less-reactive, reservoir species largely determine the ultimate effect of these trace gases on stratospheric ozone. It has been noted that model-derived concentrations of the reservoir species are particularly

sensitive to remaining uncertainties in atmospheric chemical reaction rates. For all these reasons, it was essential to measure the concentrations of reservoir species in the stratosphere.

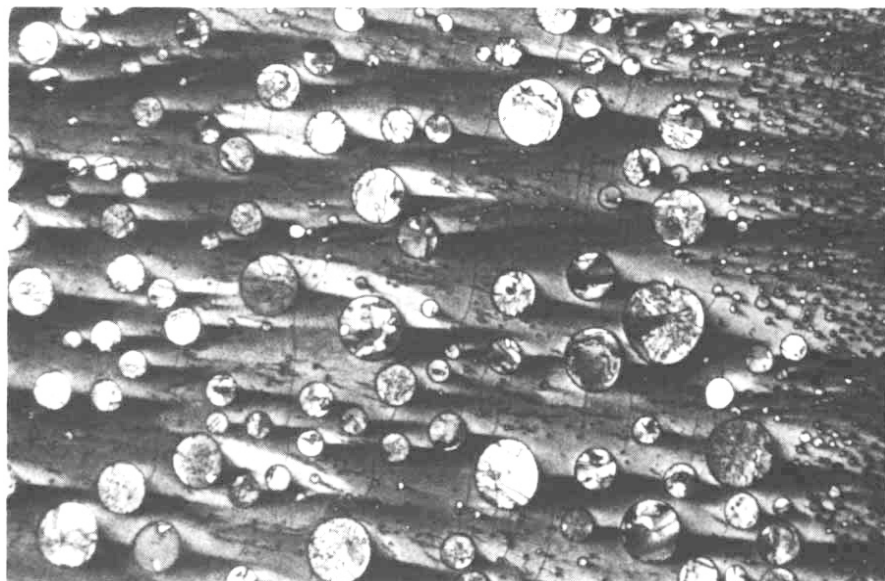
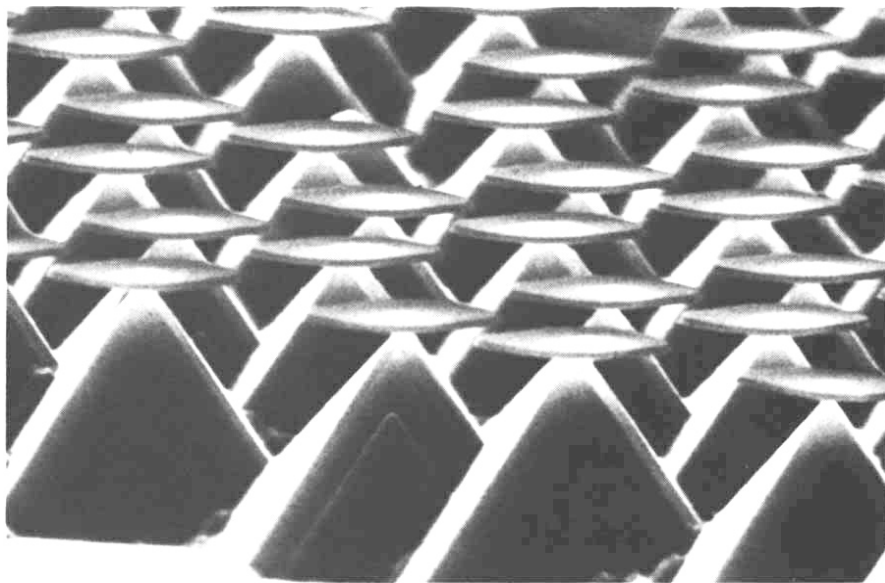
N_2O_5 has long been thought to be important in the diurnal variation of NO_2 , which reacts with ozone. The slow photolysis of N_2O_5 should produce NO_2 slowly during the day, while production of N_2O_5 should reduce NO_2 concentrations slowly in the night-time stratosphere. Observations of the sunrise-sunset differences in the NO_2 profile^{2,3} and a recent analysis⁴ of satellite-observed NO_2 at varying zenith angles have tended to verify the importance of this process. The observations by Toon *et al.*¹ are in general agreement with the expected diurnal behaviour of N_2O_5 .

Model calculations suggest that N_2O_5 concentrations are particularly large in the high-latitude winter (especially in the Arctic) and that N_2O_5 is the major portion of total reactive nitrogen in the high-latitude stratosphere (see figure). Trajectory analyses⁵ suggest that the conversion of NO_2 to N_2O_5 in the winter polar night explains the so-called Noxon cliff, in which sharp gradients in the total vertical column content of NO_2 are observed at high latitudes. Measurements of N_2O_5 concentrations in the polar winter stratosphere in tandem with further observations of NO_2 concentrations are needed to test this suggestion. In addition, heterogeneous mechanisms⁶ have been proposed for the conversion of N_2O_5 to HNO_3 in the arctic winter. This could also be tested by observations of N_2O_5 .

The observed profile of N_2O_5 in Toon *et al.*'s study is in general agreement with the expected theoretical distributions determined with models such as the Lawrence Livermore National Laboratory one-dimensional model, but a direct comparison depends on knowing the NO_2 and O_3 concentrations at the same location. As the measurements of Toon *et al.* depend on assumed profile shape, the next step is to reduce this dependence. Clearly, based on the expected diurnal behaviour and from results (see figure) that show the strong winter high-latitude gradients, observations of N_2O_5 are needed at a wide range of seasons and locations, as well as at various times throughout the day, to validate the theory. □

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These pictures won first prize in the 1985 Polaroid international photomicrography competition. The top picture of a recrystallized mixture of sulphur and ketone was taken by William E. Schadel of Raleigh, North Carolina, using a Nikon polarizing microscope and a 35mm camera (1,680 $\times$). The bottom picture of etched silicon was taken by Anita Brandes of the Gould Research Center, with a Jeol scanning electron microscope (10 $\times$).

Distant galaxies

Pushing back the redshift limit

from C. Martin Gaskell

THE search for ever more distant galaxies has become one of the main challenges of observational cosmological research. The more distant a galaxy the greater its redshift because of the overall expansion of the Universe; astronomers generally prefer to speak of the redshift of an object rather than its distance. In this issue, J.S. Dunlop *et al.* (*Nature* **319**, 564; 1986) report the discovery of a new quasar, PKS1351-018, with a redshift (z) of 3.72. And also just recently, S. Djorgovski *et al.* (*Astrophys. J. Lett.* **299**, L1; 1985) have

discovered the most distant non-quasar galaxy known. What do these discoveries mean?

Last year in these columns (*Nature* **313**, 628; 1985) I commented that the greatest known redshift for quasars had crept from 3.53 in 1974 to 3.78 in 1982, while in the same period the greatest known redshift for non-quasar galaxies shot from 0.46 to 1.82. The recent discoveries continue this trend. Djorgovski *et al.* have pushed the redshift record up to 3.218, while the quasar discovered by Dunlop *et al.* is the

second most distant quasar known. There are now more than 70 quasars with redshifts greater than 3.0, but the distribution still cuts off sharply at around 3.7.

It has required much effort to measure the redshift of PKS1351-018. Dunlop *et al.* had to obtain an accurate radio position of a compact source in an apparently blank region of the sky; then deduce from infrared colours obtained with the UK infrared telescope that the object was probably a high redshift quasar; and finally obtain an optical spectrum with the Anglo-Australian telescope. It is of interest that although PKS1351-018 is considerably fainter than the previously discovered quasars with redshifts greater than 3.5, it proved to be no more distant. Does $z=3.8$ represent some sort of 'turn-on' epoch for quasars? The suspicion is that it does, but it will take more painstaking work to establish this.

Quasars are located in the nuclei of galaxies. Does $z=3.8$ represent some critical time for galaxy formation? A normal galaxy at a redshift greater than 3 is exceedingly difficult to find but the task is much easier if one knows just where to look. Extrapolating from common experience with low redshift galaxies, Djorgovski *et al.* reason that the best place to look for a galaxy is next to another galaxy. Because quasars are located in the nuclei of galaxies they decided to use quasars as signposts or beacons for their normal companion galaxies. They chose a small sample of distant quasars and used an interference filter to look for objects emitting Lyman alpha at the redshift of the quasar. In their first 20-minute exposure with the 3-metre telescope at Lick Observatory they discovered an object emitting strong Lyman alpha near a quasar. The object has the sort of spectrum expected from a mildly active galaxy at high redshift but not the sort one sees from a quasar.

The interest of this galaxy is that it is observed when the Universe was only some 14 per cent of its present age (if we assume a critical density Universe). This galaxy is probably less than 9 per cent of the present age of galaxies depending on just when galaxies formed. If galaxies formed more recently than $z=4$ then the Djorgovski *et al.* galaxy could be a good approximation to a 'primeval' galaxy.

The nature of this galaxy is still unknown, but perhaps the most important thing is that the method, using interference filter images of high redshift quasars to find extremely distant young galaxies, works. The technique promises to expand studies of distant galaxies to redshifts as high as those of the most distant known quasars and opens the door for studies of galaxies beyond $z=3$. □

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Psychophysics

Limits of visual resolution

from Graham Martin

AT first sight we might expect that the limits of our ability to resolve an object in a visual scene constrains the precision with which we are able to judge the position of that object. However, psychophysical experiments suggest that this is not the case. Elsewhere in this issue, N.V. Swindale and M.S. Cynader provide an answer to this problem by measuring for the first time a physiological correlate of hyperacuity in the visual cortex (*Nature* 319, 591; 1986). Their results suggest that the mechanism underlying hyperacuity is relatively simple, perhaps based on the well-known properties of orientation sensitivity of cortical neurones.

It is easy to demonstrate that the smallest object that we are able to resolve in a scene, determined by measuring minimum separable visual acuity, subtends an

angle at the eye of about 1 minute of arc, but we may be able to assess the position of that object in the scene (determined by measuring vernier acuity or hyperacuity) with much greater accuracy, probably about 5 seconds of arc. The reasons for this have been discussed extensively (Westheimer, G. in *Progress in Sensory Physiology* 1; Springer, Berlin, 1970) and it has been generally agreed that these two abilities and the limits on their accuracy arise from different mechanisms in the visual system.

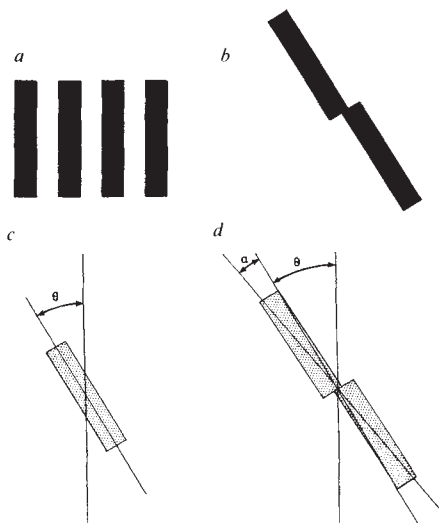
The factors limiting visual acuity seem to lie in the eye. Here the ultimate limit on resolution is dictated by the size of the photoreceptors on which the image of the world is focused. Were photoreceptors any thinner and packed more closely together than about 2 microns, then optical cross-talk between adjacent photoreceptors would degrade the information contained in the image (Miller, W.M. in *Handbook of Sensory Physiology* VII/6A; Springer, Berlin, 1979). This sort of photoreceptor spacing, coupled with the focal length of the eye, accounts well for the measured values of maximum visual acuity in various species, including man.

The mechanisms limiting hyperacuity must, however, lie not in the eye but in the visual cortex. Theoretical explanations of hyperacuity have usually involved the idea that averaging of signals from several parts of the retina occurs within the brain, together with the notion that the visual world is represented in the cortex in a much finer grain than in the retina (Barlow, H.B. *Nature* 279, 189; 1979). (There are several hundred times as many cells in the visual cortex as there are pathways entering from the eye.)

Swindale and Cynader show that if a vernier acuity type of stimulus is moved across the receptive field of a neurone in area 17 of the cat visual cortex, the response of that cell is significantly reduced compared with its response when a single bar is used (see figure). But the size of the offset necessary to cause this reduction is considerably smaller than the spatial resolution of the same cell. Thus, a single cortical cell can show properties that seem directly analogous to the distinction between acuity and hyperacuity.

Such an apparently straightforward correlation between the properties of single cortical cells and visual performance is in itself impressive. However, perhaps the most important aspect of the work is the similarity which Swindale and Cynader find between the vernier sensitivity of a cortical cell and its orientation selectivity. The properties of a vernier stimulus can be approximated by a single bar of the same dimensions but which has an orientation the same as that of a line joining the midpoints of the two bars that make up the vernier stimulus. Thus, hyperacuity may turn out to be subverted by the same or similar mechanisms to those which underlie our ability to detect differences in the orientation of a straight line, especially its deviation from the vertical. Mechanisms involving a hypothetical fine-grained representation of the visual world within the cortex may not be necessary to explain hyperacuity. But Swindale and Cynader's explanation does require that the nervous system has the ability to interpret fine fractional changes in the firing rates of neurones. Whether this is true will require further study, but such a property would simplify our understanding of how sensitivity thresholds measured psychophysically are related to properties of neurones in the visual cortex. □

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a, The type of stimulus pattern used to assess the limits of visual resolution. When using a regular grating pattern of high contrast, the smallest gap between the bars which can be detected by man subtends at the eye about 1 minute of arc. b, The limits of accuracy with which the position of an object in the visual scene can be assessed is measured by using a stimulus pattern consisting of two offset bars. The smallest offset in the alignment of these bars which can be discerned by man subtends only about 5 seconds of arc at the eye. Swindale and Cynader show that if such a stimulus is swept across the receptive field of a neurone in the cat's visual cortex, the response of that neurone is reduced compared with its response when a single bar of the same dimensions is used. The degree of reduction is comparable with that produced when the orientation of the single bar is changed from its optimal position, say θ (c) by an amount equivalent to the orientation of straight line that joins the midpoints of the two offset bars, say α (d).

Space science

First results from Spacelab 2

from Eugene W. Urban

THE Spacelab 2 mission was launched on 29 July 1985 on the space shuttle Challenger, with a crew of seven consisting of the commander, pilot, three mission specialists who operated the spacelab systems and biological experiments, and two payload specialists, both of whom were solar scientists and operated many of the experiments. What follows is an account of the post-flight science meeting held on 14 and 15 November 1985 at the Marshall Space Flight Center at which the principal investigators of the experiments (see

table) described the type, quality and status of the data and their analysis.

Spacelab 2 was originally to have been launched on 12 July 1985 for 7 days at an orbital inclination of 49.5° and a circular altitude of 385 km. This date would have given a new moon in the middle of the mission, primarily for the benefit of the infrared telescope (IRT), the plasma depletion experiment and the vehicle charging and potential (VCAP) experiment, but a technical problem caused the original lift-off to be aborted.

The launch was rescheduled for 29 July, at 1423 Eastern Daylight Time. This enabled most orbital mechanics and vehicle attitude conditions to be just as they would have been on 12 July, but coincided with a full moon rather than a new moon during the flight. However, several previously hidden active solar regions had moved around onto the solar disk, making the Sun a more interesting target for the three solar telescopes. Furthermore, the 17-day delay allowed the superfluid helium experiment (SFHE), which had suffered a component failure just before the original launch, to be repaired.

On 29 July, the launch was delayed until 1600 Eastern Daylight Time, and a technical problem left the Challenger at a lower orbital altitude (315 km) than planned, and with a significantly reduced quantity of control propellant. At first, it was necessary to cancel plans for all but two of the eight engine firings for the plasma depletion experiment and to reduce manoeuvring during fly-around operations (described below) for the plasma diagnostic package (PDP). But by careful budgeting, it was possible to recover two more firings and to add an extra day to the mission.

Plasma experiments

Data analysis from all the experiments is now in progress. The three plasma physics experiments sought, by both passive and active methods, to study the properties and behaviour of the ambient ionosphere and its effects in and around the vehicle. PDP carried 14 instruments to measure electromagnetic fields and the composition and properties of the space plasma. It operated almost continuously throughout the mission, collecting data while stowed on the Spacelab pallet; while attached to the remote manipulator system (RMS) arm and being moved over the payload bay; while in an 8-hour free flight; and while being held in a parked position on the RMS outside the bay. For the free flight, the PDP was released from the RMS and spin-stabilized at 5 r.p.m. Challenger was then flown slowly around PDP in two elliptical trajectories (maximum excursion about 300 m), after which PDP was spun down and recaptured.

Several phenomena were studied during the free flight, including the undisturbed ionospheric plasma environment; wake disturbances caused by the orbiter; vehicle disturbances propagating up and down along the Earth's magnetic field lines when Challenger and PDP lay on the same flux tube; propagation along the flux tube of the electron beam ejected by the VCAP experiment beam generator (described below); and the radio frequency field profile of the orbiter's radar and communication antenna. A particularly interesting observation was made when the VCAP electron beam intercepted the PDP. The plasma wave behaviour at PDP

Spacelab 2 experiments and their principal investigators

Ejectable plasma diagnostics package	Louis Frank, University of Iowa
Vehicle charging and potential experiment	Peter Banks, Stanford University
Plasma depletion experiments for ionospheric and radio astronomical studies	Michael Mendillo, Boston University and Paul Bernhardt, Los Alamos National Laboratory
Solar magnetic and velocity field measurement system/solar optical universal polarimeter	Alan Title, Lockheed Solar Observatory
Solar helium abundance high resolution telescope and spectrograph	Guenther Brueckner, Naval Research Laboratory and Alan Gabriel, Rutherford Appleton Laboratory
Coronal helium-abundance spacelab experiment	J. Leonard Culhane, University College London
Solar ultraviolet spectral irradiance monitor	Guenther Brueckner, Naval Research Laboratory
Small helium-cooled infrared telescope	Giovanni G. Fazio, Smithsonian Astrophysical Observatory
Hard X-ray imaging of clusters of galaxies and other extended X-ray sources	A. Peter Willmore, University of Birmingham, England
Elemental composition and energy spectra of cosmic ray nuclei	Peter Meyer and Dietrich Mueller, University of Chicago
Properties of superfluid helium in zero-gravity	Peter V. Mason, Jet Propulsion Laboratory
Vitamin D metabolites and bone demineralization	Heinrich K. Schnoes, University of Wisconsin
Gravity-influenced lignification in higher plants	Joe R. Cowles, University of Houston

bore a strong resemblance to earlier satellite observations of natural aurorae, indicating the value of active *in situ* plasma experiments.

The VCAP experiment included passive charge, current and plasma probes and a low-energy electron-beam generator, which operated many times in both continuous and pulsed modes, emitting 50 or 100 mA beams at 1,000 V. The plasma environment and natural vehicle charging caused by the orbiter's interaction with the ambient plasma were measured frequently. Local effects of beam emission were measured by VCAP sensors; during the two PDP flux-tube connections the beam and its remote effects were detected by the free flyer. Data quality and quantity were excellent, except for low light level television and photographic images of the beam, which were overwhelmed by the bright moonlight.

The plasma depletion experiment consisted almost entirely of ground-based radio and optical observations of the large electron-depletion regions created in the ionosphere by the exhaust products from the firing of the orbiter manoeuvring system engines over specified ground observatories. Although the propellant shortage and low altitude limited the number of engine burns, two were made over Millstone Hill, Massachusetts (one in daylight and one at night), and one each at night over Hobart, Tasmania and Arecibo, Puerto Rico. Significant ionospheric holes developed and were observed as they slowly recovered. Incoherent scatter radar measured the size and density of the electron-depletion regions above Millstone Hill and Arecibo, and night-time observations of the 630-nm airglow from these two burns were made despite the bright moonlight. The ionospheric radio cutoff frequency at Hobart dropped

appreciably, permitting receipt on the ground of normally blocked radio astronomy signals.

Solar experiments

The three solar and one atmospheric physics experiments were mounted and co-pointed on the instrument pointing system (IPS) provided by the European Space Agency. The IPS was on its maiden flight, and could itself be considered to be an experiment. When the IPS was under pointing control of the solar instruments and later, when under control of its own optical sensor package, the required quiescent pointing stability of about 1 arc s was achieved.

The solar optical universal polarimeter (SOUP) instrument was designed to produce stable high-resolution white-light images of the Sun's surface granulation and to generate narrow-band filtergrams in polarized light to exhibit magnetic and velocity fields. Because of an unexplained hardware problem, the instrument could not be operated until the seventh day of the mission. A total of only 18 hours of solar observing by SOUP was possible, but the quality of the photographic and video data obtained is splendid.

The observing time was devoted to the diffraction-limited white-light imaging system. The video images were viewed in real time by the crew and from the ground; more than 6,000 frames of photographic film were exposed in long rapid sequences of stable high-resolution images of selected solar features. Image stability was much better than 1 arc s, thanks to an internal image motion compensation system. These filmed sequences show the evolution of extended structures and very small, previously unobservable features. Limited filtergram data were collected, but have not yet been studied.

The high-resolution telescope and spectrograph (HRTS) instrument consists of an 0.5–1.0 arc s resolution telescope, a hydrogen Lyman alpha (H_{α}) full disk system with both video and photographic recording, and an ultraviolet spectrograph and a full-disk ultraviolet spectroheliograph, both recording on photographic film. The H_{α} video image was available on board and on the ground for target selection. The objective was to study rapidly moving solar chromospheric features with high spatial and temporal resolution. Although HRTS experienced some serious thermal problems, it accomplished most of its objectives and recorded images on most of its photographic film: more than 1,300 spectroheliograph and 30,000 spectrogram images were obtained. Spectroheliograph sequences at the solar limb show details of small, rapidly evolving features which protrude into the corona. Rapid spectrograph sequences display small, short-lived, highly turbulent structures, and spectra taken in steps across the disk show larger and more stable features.

The scientific objectives of the coronal helium abundance spacelab experiment (CHASE) were to measure accurately the abundance of helium in the Sun relative to hydrogen by means of an extreme ultraviolet spectrometer looking at the intensities of several specific solar emission lines. CHASE accomplished almost all of its planned observations and produced very high quality data. By scanning both the spectrometer slit and the grazing incidence mirror, the data have generated maps at moderate resolution of much of the visible disk and the corona in several narrow spectral bands simultaneously. The maps will permit accurate determination of the helium to hydrogen abundance, and show both temperature and density in the solar atmosphere.

The solar ultraviolet spectral irradiance monitor (SUSIM), an ultraviolet spectrometer from an earlier shuttle mission and destined to fly repeatedly, is designed to measure long-term variations in the ultraviolet flux from the Sun. An internal calibration source and comparison spectrometer permit absolute flux to be measured as a function of wavelength. Despite some overheating, it accomplished more than 50 high-resolution spectral scans of the solar ultraviolet output.

High-energy experiments

The cosmic-ray nuclei experiment instrument, weighing 1,968 kg, is the heaviest scientific instrument ever carried into space. It was designed to observe and identify primary cosmic-ray particles out to energies of several TeV per nucleon, using gas Cerenkov counters and a transition radiation detector. The instrument operated smoothly throughout the mission, collecting on the order of 70 events per second for a total of 40 million cosmic-

ray particles, about 0.1 per cent of which were at energies greater than 40 GeV per nucleon. Of those, only one in 50 is expected to have an energy greater than 400 GeV per nucleon. Much analysis will be necessary before the energy spectrum is known and the particles identified atomically.

The X-ray telescope consists of two similar X-ray imaging instruments, each using a shadow mask and a multiwire proportional counter. The telescopes are carried on a two-axis pointing mount. Designed to generate images of clusters of galaxies in the 2–23 keV range, the pair of instruments, with angular resolutions of 3 and 12 arc min, observed many targets in very small increments of time. A total of at least 4 hours of data were gathered on each of six primary targets including the Perseus, Centaurus, Coma and Virgo clusters, the Milky Way galactic centre and calibration sources; several secondary targets were also observed for shorter times. Even with analysis of limited data sets, interesting maps of X-ray emitting galaxy clusters are beginning to emerge. One result is that there seems to be no X-ray source at the galactic centre in this energy range.

Infrared telescope

The scanned infrared telescope (IRT) consisted of a highly baffled hersehian telescope and 10 detectors covering wavelengths from 2 to 120 μm . The detectors and telescope were cooled to very low temperatures by helium gas delivered from a separate superfluid helium dewar so that the detector temperature was 3–1 K. When IRT observations began, a strong background saturated the intermediate wavelength bands, limiting astronomical data to the longest and shortest. Observations in these bands along the galactic plane complement earlier maps from the infrared astronomical satellite. The nature and variability of the apparent background is being studied, but may be partly explained if the water vapour density around the orbiter is higher than expected.

When the PDP was held in front and to one side of the IRT so that its base intercepted the ionospheric gases flowing past the orbiter, spacecraft glow was expected to be seen in the short wavelength channel. None was found, either because there was no glow at that wavelength or because an extended glowing volume enclosed the entire orbiter.

Performance data from the superfluid helium cooling system of IRT will be of value in the design of future cooled instruments in space. In one important test on the superfluid helium, venting from the dewar was stopped for several hours, causing the liquid to warm to the normal phase. When the venting was resumed, the liquid cooled to the superfluid phase

again, confirming our ability to control a large volume of liquid helium in zero gravity, even when it is not superfluid.

Additional experiments

The SFHE was designed to study the thermal and fluid properties of bulk liquid and the fundamental interactions between the normal and superfluid constituents of very thin films of liquid. Small experiment modules within a 100-litre dewar vessel provided a stable temperature environment between 1.5 and 2.2 K. Within the bulk fluid module an array of thermometers detected the thermal response of the liquid helium to controlled heat inputs. An array of liquid/vapour sensors measured the liquid motion in response to both deliberate mechanical disturbances and periods during which equipment and crew remained silent. Flight data are being analysed. Within the quantum surface wave module, five toroidal channels, each with a different thickness of superfluid film, were excited by small heat pulses. Propagation of the pulses along the films was observed under many conditions. The results will be related to theoretical predictions.

Blood samples were taken from two payload specialists and two of the mission specialists before the flight, twice during the mission and after the flight. Analysis of the plasma, which is now in progress, will seek to add to our knowledge of spaceflight effects on vitamin D metabolism, calcium balance and bone demineralization.

Conclusions

Despite several problems, the scientific returns of the mission have clearly been very great. During the mission, the experimenters monitored and recorded portions of the data in real time. Complete data records have now been distributed for an analysis period which will take at least a year.

This summary covers only a few details of the Spacelab 2 mission but should give some idea of the quality and quantity of scientific information that was collected. The success of the experiments derives largely from the intensive efforts of the operations and science team at the Payload Operations Control Center at the NASA Johnson Space Center, Houston.

NASA is planning to fly a major portion of Spacelab 2 again in about two years time in the Sunlab/Dark Sky mission. This will allow investigators to improve their data-gathering techniques and considerably to increase the amount of astrophysical data that made Spacelab 2 such an important scientific payload. □

Eugene W. Urban was the Spacelab 2 mission scientist and is at the NASA George C. Marshall Space Flight Center, Huntsville, Alabama 35812, USA.

Accidental human vaccination with vaccinia virus expressing nucleoprotein gene

SIR—Infectious vaccinia virus recombinants that express genes of other pathogenic agents have been proposed as candidates for live vaccines¹⁻⁹. In several cases, vaccination has been shown to effectively immunize and protect experimental or domesticated animals against a virus challenge. Here we report the physical and immunological responses in a human accidentally vaccinated while vaccinating mice with a recombinant, derived from the WR laboratory strain of vaccinia virus, which expresses the vesicular stomatitis virus serotype Indiana (VSV-IND) N protein⁸. In this incident, the vaccinated human and experimental mice had the same specific antibody response.

Vesicular stomatitis virus (VSV) is an RNA-containing, bullet-shaped, enveloped rhabdovirus that causes a highly contagious vesicular disease of cattle, horses and pigs. In humans, vesicular stomatitis (VS) causes influenza-like symptoms¹⁰. Epizootics of VS in US cattle occurred in 1916, 1949, 1963, 1982 and 1985. Because VS is initially indistinguishable from foot-and-mouth disease, herds with VS must be quarantined at great cost to the livestock industry.

We are investigating the immunogenicity of infectious vaccinia virus recombinants that express the G and N proteins of vesicular stomatitis virus (VSV)^{8,11}. While one of us (L.J.) was injecting mice with an infectious vaccinia virus recombinant expressing the N gene of VSV (v38), a small cut on the right ring finger was inoculated with a drop of virus (3.0×10^6 PFU/0.05 ml). Although L.J.'s hands were washed

immediately, the finger was slightly reddened and swollen 4 days later, as were similar lesions on the vaccinated mice. The swelling progressed from the base of the fingernail past the first joint of the finger, restricting movement of the joint. On day 8, the right axillary lymph node became swollen; the lymph node and finger swelling began to subside approximately 12 hours later. On day 10, the finger swelling had completely disappeared, but a single localized pock lesion appeared in the reddened area, coinciding with the appearance of similar lesions in the mice. The lymph node swelling disappeared by day 12. The lesions were completely healed by day 25 in both L.J. and the mice.

During the infection, L.J. did not experience fever or general malaise. The mildness of the infection is notable, despite the fact that a non-vaccine strain (WR) was involved in this exposure. The mildness of the infection could be related to a smallpox vaccination that L.J. had been given some 30 years before the accident, or to the attenuation of the vaccinia virus recombinant by the insertional inactivation of the thymidine kinase gene, as recently demonstrated¹².

Sera from L.J. and the mice were evaluated by immunoprecipitation of ³⁵S-methionine-labelled extracts of baby hamster kidney-21 cells infected with either VSV-IND or the VSV serotype New Jersey (VSV-NJ) and analysed on SDS-PAGE. There was only specific binding to the N protein of VSV-IND (Fig. 1). All sera were positive by ELISA

against whole VSV-IND and negative against whole VSV-NJ. The absence of binding to the G protein of VSV-IND indicated that L.J. had not been infected with VSV. As expected, the sera did not have serum-neutralizing antibodies for VSV-IND or VSV-NJ since the N protein does not cause the production of serum-neutralizing antibodies. The neutralization titres to vaccinia virus of serum taken from L.J. 1 month and 4 months postinfection were 256 and 64 respectively, indicating that this was a recent infection.

These data demonstrate that the vaccinia virus recombinant can produce specific antibody to a non-vaccinia virus protein in humans as well as in mice.

L. JONES*

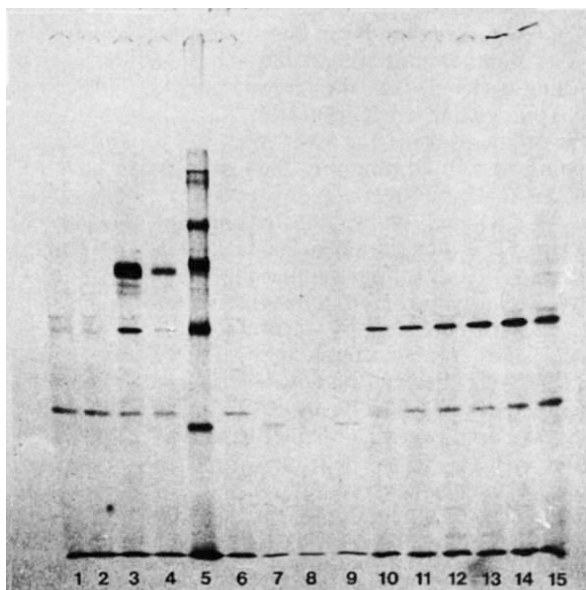
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Fig. 1 An autoradiogram of SDS-PAGE analysis of sera from L.J. and vaccinated mice. The sera were immunoprecipitated as described by Kessler¹³ with a lysate of ³⁵S-methionine-labelled cells infected with either VSV-IND or VSV-NJ with an MOI of 4. SDS-PAGE¹⁴ was performed using a 10% acrylamide running gel, fixed, dried, and allowed to expose X-OMAT film for 2 days. Antigens: lanes 1–4, 6, 7, and 10–15, VSV-IND; lanes 8 and 9, VSV-NJ. Sera: lane 1, from a mouse bearing a myeloma (P3X63) for fusion; lane 2, normal BALB/c mouse; lane 3, mouse anti-VSV-IND; lane 4 is mouse anti-VSV-NJ; lane 6, mouse anti-v50 (a vaccinia recombinant bearing the gene for the VSV-NJ G protein); lanes 7 and 8, normal human; lanes 9 and 10, from L.J.; lanes 11–15, from five different BALB-c mice infected with v38. Lane 5 contains ¹⁴C relative molecular mass standards (200K myosin, 100K/92.5K phosphorylase b, 69K bovine serum albumin, 46K ovalbumin, 30K carbonic anhydrase, 14.3K lysozyme).



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Malaria — mitogens or super antigens?

SIR—The induction of a variety of immune responses by malarial infections led Greenwood¹ and Wyler² to propose the existence of a malaria mitogen. The activity of extracts of blood cells infected with *Plasmodium falciparum* and *Plasmodium berghei* has been studied using blast transformation of human peripheral blood lymphocytes³⁻⁸ and rodent splenocytes⁹⁻¹¹. The results ranged from the demonstra-

tion of antigen-specific stimulation of previously sensitized lymphocytes^{3,8} to mitogen-like stimulation of both sensitized and non-sensitized lymphocytes^{4,6,7,9}. A close look at the literature and careful attention to the results obtained raises the question whether the stimulatory material in malaria parasite extracts is, in fact, a mitogen.

The points that have been put forward to support a mitogenic nature of the active factor include: (1) Ability to stimulate non-immune^{4,7,10,11} and cord blood^{3,7} lymphocytes; (2) the observation that a large number of immunological specificities are involved in malaria-induced autoimmunity¹²⁻¹⁸ and (3) the belief that only a small proportion of the high serum immunoglobulins in infected individuals contains antibody activity specific for malaria parasite^{4,7,19}.

Results consistent with the possibility that the response to malaria extracts by normal spleen cells represents primary *in vitro* sensitization have been obtained⁹. This response exhibited delayed kinetics and reduced magnitude compared to the response by immune cells. The intense activity of the reticuloendothelial system during malarial infection¹⁹ may result from stimulation by highly potent antigens from the parasite. This high potency of antigenic materials from plasmodia may be responsible for the response of cord blood lymphocytes. There is evidence against a functional immaturity, and for a lack of adequate triggering of surface receptors on cord B lymphocytes²⁰. Also, the human fetus is able to respond immunologically to intrauterine infections²¹⁻²⁴.

While autoantibodies directed against a number of autoantigens have been described in malaria, an abnormal frequency of only anti-nuclear antibodies was found in a recent survey²⁵ carried out in a malaria endemic region in Upper Volta. Thirteen other autoantibodies studied had a normal frequency. This selective increase in the frequency of one autoantibody cannot result from a nonspecific polyclonal mitogenic activation. However, Phanuphak *et al.*²⁶ reported results associating acute malarial infection with high incidence of autoantibodies of both nuclear and smooth muscle antigens. Another factor that may operate in the pathogenesis of autoimmunity could be repeated stimulation of the reticuloendothelial system with parasite antigens cross-reactive with normal tissue antigens²⁷.

Although there is evidence to show that only a fraction (6-11%) of the high serum immunoglobulin levels in malaria is specific malaria antibody¹⁹, there seems to be no report indicating what proportion of the total immunoglobulin produced in response to malarial infection this fraction represents.

In the light of the foregoing, is it not

more appropriate to talk in terms of highly potent (or "super") antigens rather than mitogens in malarial infection?

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The riddle of the magnetic doors

SIR—The Wei and Ching rivers in the northwestern part of China have their sources in Kansu province and flow into the Yellow River (Huang Ho). This river basin has been in fact the cradle of ancient Chinese civilization. It was known that the Wei is rather muddy and the water of Ching is clear; when they join together, the border line can still be seen. Sian on the south bank of the river Wei was a flourishing city during the Ch'in dynasty about 2,200 years ago.

As is generally known, the first emperor of the Ch'in dynasty (221-206 BC), Shih Huang Ti, successfully unified the country. Recently near his burial place, not far from Sian, a brigade of terracotta soldiers and horses has been found and carefully excavated. The total number of life-size figures is about 6,000. Besides many metal weapons and swords, a carriage hauled by four horses made very delicately of bronze has also been discovered. A large museum has now been built at the spot.

There has long been a tradition that the door of the tomb of Ch'in Shih Huang Ti could not be cut through by iron swords because iron was attracted to it. This may seem incredible but the records of the newly found *Yung-Lo Ta Tien* are worth

mentioning. This was the world's largest known encyclopedia, and was compiled in China under the direction of the Yung-Lo emperor in 1406 (for details see *Encyclopedia Britannica* x, 847; 1980). According to Chinese literature the work contained 22,870 chapters. It was unfortunately scattered in the course of time, but a remnant of this encyclopedia, totalling 730 chapters was published in China in 1960.

Among the palaces of Ch'in Shih Huang the magnificent and splendid Ah Fang Palace was described by the famous poet Tu Mu (AD 803-853) whose rhymed description has attracted thousands of Chinese scholars. Much to our interest, another volume of the scattered *Yung-Lo Ta Tein* has been recently discovered in Yeh Hsien, a district in Shantung province. The fact that the doors of the Ah Fang Palace were built with magnetic stone is reported in this volume. We translate the description in the following passage: "There were gates of magnetic stone in Hsien-yang (a city 20 km north-west of Xian), 15 li (about 5.5 km) south-west of the city. On the east and west there were bridges leading to the north gate of the Ah Fang Palace. The gate was built of magnetic stone. Warriors wearing iron armour were detained or attracted and could not pass through".

An article entitled "The Western Capital" written by Wei Su was also quoted in this volume. It states that the gates of Ah Fang Palace were made of magnetic stone so that men with swords could not enter.

Some scientific facts recently found may perhaps be connected with the above literature. A few years ago a member of the commune near Sian with modern knowledge of magnetic ferrites — which are ferrimagnetic oxides used in making devices for high frequencies in modern electronics — wrote and told me that the natural river sand from the Wei River can be sintered and used as ferrite materials with good magnetic properties. He sent me about a kilogram of the river sand from Wei. I have had the sample analysed in our laboratory. The material possesses the following chemical composition in weight percentages: Fe-69.29; Si-0.58; Mn-0.14; MgO-0.25; W-1.0; Ti-0.55; Cr-0.20; and Al-0.30.

The oxides are black in colour. As they are highly magnetic, it may be concluded to be a mixture of magnetic Fe₃O₄, ilmenite FeTiO₃ and other oxides including WO₃ and FeCr₂O₄. The quality of the material is not inferior to the synthetic oxides used in the manufacture of ferrites in modern industry.

Unfortunately we have not yet found any direct evidence that this magnetic sand was used in ancient times.

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A mind for investigation

Donald Broadbent

The Mind's New Science: A History of the Cognitive Revolution. By Howard Gardner.

Basic Books: 1985. Pp. 423. \$22.50.

To be published in Britain in June by Harper & Row, £15.95.

A QUIET change has come about over the past 50 years. We now have a different mode of attack on problems of "higher nervous function", or "the mind" or "behaviour". In the 1930s, the alternatives were to use concepts from the nervous system (particularly popular with physiologists, though to some extent with psychologists also); or to use refined terms from everyday language (particularly popular with philosophers); or to use direct descriptions of what people and animals were observed to do (particularly popular with psychologists, anthropologists and linguists).

Nowadays we have a fourth alternative. We can talk about operations performed on abstract symbols, where each symbol could be represented in a variety of physical ways. We can then discuss the differences between different kinds of representation and process, without for the moment knowing what neural events correspond to each symbolic description. Indeed, we can discuss kinds of intellectual operation that do not occur in human beings at all; and because it does not matter what the physical substrate of the processes may be, we can build machines to undertake them. These machines are of course computers, and therefore the five classic disciplines concerned with "the mind" have now been joined by computer science.

The change has been a quiet one, and cynics may say that this is because it is not visual. It does not make good television programmes. Another reason may be that the effects are spread over such wide fields of knowledge that it is hard to see what is happening; even people working in one of the six disciplines usually have only a slight knowledge of what is going on in the others. There has been no accessible account of the whole domain and all of its ramifications. *The Mind's New Science* is an attempt to provide such a broad view; briefly, it does so and to my knowledge has no competitor.

That alone must give the book a big claim on those wanting to find out what is meant by "cognitive science". But why should anyone do so? Well, if for no other reason, because large amounts of research funding are involved. In the past decade, the Alfred P. Sloan Foundation alone has committed twenty million dollars to the area (it was in fact the Sloan Foundation that supported the writing of this book).

Other agencies, though less dedicated to the field, have added substantially to the total resources. There is a clear and visible social movement under the cognitive science banner, and scientists in general ought to make up their minds about it.

Next, the practical impact is likely to be considerable. If the field is called Information Technology, that may be more obvious. But the claims of cognitive science go further than the transformation of ways of life by the use of complicated devices. Educational methods, and social institutions, may well be deeply affected without any change in the physical technology that is employed.

Above all, the reason for finding out about these developments is because they change our view of ourselves. That may be why they sometimes arouse such strong emotions — the enthusiasm (dare one call it hype?) of some cognitive scientists on the one hand, and the counter-position exemplified by John Searle's Reith Lectures of 1984 on the other. The question is whether one is dealing with a purely temporary excitement that will expire when the fashion changes, a kind of Mesmerism or Lamarckism; or are we witnessing a lasting change such as the arrival of Darwinism? Anybody with intellectual curiosity ought to wish to find out.

Gardner has set about his task by dividing the book into three sections. In the first, the earliest roots of the movement are described in an integrated way, covering the 1950s and early 1960s. In the second, each of the six fields is discussed separately, going through from the very beginning to the present day, and outlining the main ideas of certain prominent figures. The variety is enormous, from Dennett on the legitimacy of ascribing beliefs and desires to a machine to Kandel on synaptic changes during learning in the sea-slug. On the way we pass (to take just one example from each of the other four disciplines) Sternberg on the interpretation of human reaction-times, Chomsky on transformational grammars, Newell and Simon on the role of condition-action rules in computer programs for solving problems, and Levi-Strauss on kinship-systems in a variety of societies.

In the third section, the multi-disciplinary approach is illustrated by taking four specific areas of contemporary debate: visual perception, imagery, the formation of conceptual categories and

classes, and reasoning. As those in the field would expect, the names to figure prominently in these areas are Marr and Gibson, Kosslyn and Pylyshyn, Rosch and Berlin, Johnson-Laird and the double act of Kahneman and Tversky. Frankly, as examples of cross-disciplinary work they reveal the nakedness of the land; no one area involves all six disciplines, and several disciplines appear in only one area each.

How well does Gardner cover this vast area? Probably better than anybody else could have done. Each field is described clearly and attractively, and, in those I know least well, I felt I had learned a lot. In the areas I know best there are errors I would describe as howlers, and I suspect that may be true in those I do not know. But that would always be true with a book of this kind. One should compare it, not with writing for specialists, but with science journalism. By that standard it rates very highly. The choice of topics and authors is good, the amount of detail about right and the writing superb.

Will it then make friends and influence people? My feeling is that the impact will be different on readers of different backgrounds. For people in the humanities, or the educated public, it should go down well. The doubt arises about the effect on scientists. The whole style of the book is to emphasize personalities, places and occasions; particular small-scale meetings, confrontations or reports. There are very few factual findings or even theories that are more than sketched in. The description is of a social movement rather than of a body of data.

Perhaps symptomatically, Gardner chides neuroscientists for their neglect of the cognitive approach. He also expresses doubt about the ultimate separate existence of psychology. These however are the disciplines with a particularly strong empirical, as opposed to theoretical, content. It is important to remember the difference between fact and theory. A computer can execute intellectual operations that human beings cannot, just as mathematicians can discuss worlds that are not physically true. We need the separate disciplines of physics and mathematics to distinguish what is, and what might be. In the same way it is no disparagement of artificial intelligence to say that it is an equal to, not a replacement for, neuroscience and psychology.

Conversely, Gardner weakens quite needlessly the case for computational models by describing the computer as a metaphor for human cognition. The whole point of the line of argument that descends from Turing is that the same abstract set of operations can be implemented in a computer or in a person; the one is no more a metaphor for the other than a falling apple is a metaphor for the Solar System. ►

It seems, in fact, that Gardner likes best the play of ideas, discussed and adopted for their inherent appeal rather than for their truth or falsehood. This intellectualist bias may play a part in his criteria for deciding whether a particular line of work falls within the area of cognitive science. He gives a prominent place to an alleged principle that such work should not consider affect, emotion or context. There is however an enormous body of research on changes in human cognition as a function of clinical conditions, of personality, of temporary mood and of external circumstances such as the working environment. The techniques and concepts used are indistinguishable from those in the rest of cognitive psychology, but the "cognitive science" movement tends not to see them as part of the in-group. Leaving out all such work on principle has some curious side effects, however. The work of Gordon Bower of Stanford is not mentioned; it would be difficult to do so without entering Gardner's self-imposed prohibited area. Other authors such as Bartlett and Bruner (and indeed myself) are quoted prominently but without mentioning the fact that quite a lot of their work is on affect or context.

Immunologists, rheologists or acousticians, then, are likely to be worried by the minor role played by data, and will notice immediately that the six disciplines do not in fact use the *same* concepts in the way that different biological disciplines use the concept of the genetic code. Their common sense may rightly tell them that cognition without context and affect is unlikely to prove adequate; and a general distaste for bandwagons may make them resistant to the whole euphoric style. Such a backlash would be a pity. Within each of the disciplines there is a hard core of professionalism that has indeed made important advances, and will do so in the future. There is no true integrated subject of cognitive science, but there is a wide scatter of separate advances in the study of cognition which other sciences could do well to study. It is unlikely that anybody will produce a better menu than Gardner; but the menu should not be confused with the banquet. □

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New in paperback

- *R.A. Fisher: The Life of a Great Scientist* by Joan Fisher Box. Publisher is Wiley, price is \$22.50, £23. For review see *Nature* 278, 784 (1979).
- *The Quantum World* by J.C. Polkinghorne. Publisher is Pelican/Princeton University Press, price is £2.95, \$6.95. For review see *Nature* 308, 783 (1984).
- *Supermanifolds* by Bryce De Witt. Publisher is Cambridge University Press, price is £12.95, \$24.95. For review see *Nature* 313, 329 (1985).

Fictional alliance in subjugation

W.F. Bynum

The Old Brown Dog: Women, Workers, and Vivisection in Edwardian England. By Coral Lansbury. *University of Wisconsin Press: 1985. Pp. 212. \$23.50, £23.50.*

IN 1903, two Swedish medical students published a little book entitled *Shambles of Science*. It contained extracts from diaries kept of physiology classes they had attended at University College and other institutions in the University of London. Its purpose was to fan antivivisection sentiment, partly by arguing that the terms of the 1876 Act (which regulated, and still regulates, scientific research requiring vivisection) were being regularly and flagrantly contravened by physiologists. On the basis of the evidence presented by the Swedes, Stephen Coleridge, honorary secretary of the National Antivivisection Society, publicly accused Dr William Bayliss of University College of breaking the law. Bayliss sued Coleridge for libel and won substantial damages.

Three years later, Battersea Council erected a statue:

In Memory of the Brown Terrier Dog Done to Death in the Laboratories of University College in February, 1903, after having endured Vivisection extending over more than two Months and having been handed over from one Vivisector to Another Till Death Came to his Release. Also in Memory of the 232 dogs Vivisected at the same place during the year 1902. Men and women of England, how long shall these Things be?

Deliberately provocative, the statue disturbed many at the University of London and led to a series of attempts by medical students to destroy it. Some three years later, a new Council in Battersea quietly removed it. On 12 December 1985 a new statue to the "Old Brown Dog" was unveiled in Battersea Park, complete with a repetition of the original plaque. *Plus ça change.*

The "Brown Dog Riots" are part of the lore of medical research in Britain, collectively remembered by physiologists and antivivisectionists alike. They provide the starting point for Coral Lansbury's powerful new book. She provides some additional detail about the circumstances surrounding the brown dog, particularly on the extent to which the publicity generated by the libel suit, statue and riots created a brief alliance between two traditionally disparate groups, working men and middle-class women.

In practice, however, Lansbury is less concerned with social realities than with symbolism, less preoccupied with actions than with emotions. The core of her book

develops a thesis of startling bravado. It is this: that there is a symbolic identity between an experimental animal on a laboratory table, and a woman undergoing a gynaecological examination. Furthermore, both are inexorably linked to another sphere of sadistic fantasy, pornography, especially the favourite Victorian scenario in which whipping and birching are an important kind of foreplay:

The distinction between the vivisector's experiments and the episodes of the pornographic novel is that in the latter there is never any question of failure, for the victims of the riding

IMAGE
UNAVAILABLE
FOR
COPYRIGHT
REASONS

Emotional appeal — the new "Brown Dog" statue in Battersea Park, London, which was unveiled on 12 December last year.

master always end as lascivious, demonic servants of the whip, but the animals resist to the bitter moment when they are released by death [p.127].

We thus have a neat equation in which victim—animal—woman is subdued by pornographer—vivisector—gynaecologist. The latter three are, quite simply, dirty words.

Much of Lansbury's "evidence" is literary (she is a professor of English) and much of the volume is devoted to analysing Victorian fiction: pornography like *Birch in the Boudoir*, animal stories like *Black Beauty* (a particular favourite of hers) and novels by Wilkie Collins, George MacDonald and H.G. Wells. Her excessive reliance on literary exposition detracts from the historical persuasiveness of the exercise, as does the caricature of experimental medicine which vitiates the book.

Professor Lansbury is not required to like science, but if she wishes to write about it she does have a certain basic responsibility to her historical sources. My heart sinks when I see someone refer to *Origin of the [sic] Species* (p.154), as I think it is safe to assume that this person has not read, much less digested, Darwin's great work. My confidence is not strengthened when I discover Robert Koch referred to as a "physiologist", Claude Bernard (1813–1878) and Ferchault de Réaumur (1683–1757) described as "contemporaries", or Sir John Scott Burdon Sanderson rechristened "James". For Lansbury, as for nineteenth-century antivivisectionists, Claude Bernard was the chief spokesman for the new and vicious priesthood, intent on replacing the old values with the

new gospel of materialism and science.

To give Professor Lansbury her due, she is aware that she has forsaken the subtle greys of historical analysis in favour of the blacks and whites of polemic: "Where emotions are the subject, quantification and statistics have proved notably deficient, and the historian is wise to defer to the poet and novelist". I doubt it. Elsewhere, she lays her cards on the table: "I know of only one law in history, and that is that people will always believe what they want to believe, despite all evidence to the contrary". This is profoundly depressing, but Lansbury's book makes me think it may be true. □

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Mogi's doughnuts

Chi-Yu King

Earthquake Prediction. By Kiyoo Mogi. *Academic: 1985. Pp. 355. \$64, £53.50.*

A VAST amount of information on earthquake prediction has accumulated in recent years, and has been published in several different languages in many different journals and reports. Under such circumstances, it has proved impossible for any individual to write a book on the subject that is coherent, comprehensive, up-to-date and without parochialism or personal bias. Mogi is not excepted.

The book has been translated from Japanese, with slight modification. It should, perhaps, have retained its original title, *Earthquake Prediction — A Review of the Japanese Programme*, for it mainly deals with material from Japan and Mogi's own research. Some of the older information has been covered by T. Rikitake in *Earthquake Prediction* (Elsevier, 1976), but much of the book contains newer data collected over the past decade. The translation retains a Japanese flavour, and some of the location maps are not labelled in detail so a familiarity with Japanese geography is helpful. A few technical terms (for example hypocentre, epicentre; dip, tilt) are used without discrimination. The quality of printing, however, is very good.

Mogi begins with a general discussion of the feasibility and physical basis of earthquake prediction, stressing the importance of crustal heterogeneity as a governing factor of precursor events and commenting on the success and failure of the dilatancy models and possible usefulness of the time-predictable and slip-predictable models. He then describes prediction-related studies in various disciplines, covering crustal deformation, patterns of occurrence of smaller earth-

quakes in space and time (including doughnut-shaped seismic gaps), seismic-wave velocity changes, electromagnetic variations, groundwater and geochemical changes, active fault movement and laboratory observation of rock fracture. The coverage is not even-handed; much space is devoted to crustal movement and seismicity, little to hydrological and geochemical studies, and none at all to observations of anomalous light, fog and animal behaviour, or to engineering and socio-economic effects.

Next comes a detailed review of interdisciplinary observations of significant earthquakes that have occurred since 1965, when the Japanese national programme on earthquake prediction began. Included here are accounts of the 1973 Nemuro-Hanto-oki earthquake (magnitude 7.4), the 1978 Miyagi-ken-oki earthquake (7.4), the 1983 Japan Sea earthquake (7.7), and the earthquakes that occurred along the Yamasaki fault and in and around the Izu region. Finally, Mogi describes the Japanese prediction efforts in the Tokai region, where a large earthquake is anticipated, and around Tokyo, and rounds things off with a discussion of strategy of long-term to short-term predictions.

The book contains no mathematics and relatively few technical details, and is thus suitable not only for experts but for non-specialists looking for a coherent and up-to-date account of Japanese work on earthquake prediction. Those who wish to know more about research going on elsewhere (notably in the Soviet Union, China and the United States), or in disciplines not well represented, will need to consult the proceedings of international symposia such as those published by the American Geophysical Union in 1981 and by Terra Scientific Publishing for UNESCO in 1984. □

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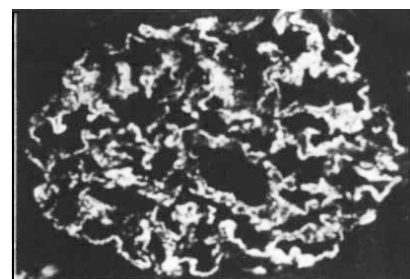
Looking for antigens

Julian Heath

An Atlas of Immunofluorescence in Cultured Cells. By Mark C. Willingham and Ira Pastan. *Academic: 1985. Pp. 186. \$35, £31.*

IN recent years the use of fluorescently-labelled antibodies to localize antigens in cells and tissues has been a prominent research tool for the cellular and molecular biologist. The compilation of an atlas of immunofluorescent images was therefore a good idea. But a truly comprehensive atlas would need to contain as many maps as there are antibodies, and would be a very large tome indeed, so Willingham and Pastan have restricted themselves to the terrain with which they are most familiar. Their small volume contains images of cultured fibroblastic and epithelioid cells stained with antibodies to 26 cytoskeletal, membrane, nuclear and viral antigens, many of which they have used in their own research.

Although there is a 12-page introduction discussing specimen preparation for immunofluorescence, the overall feeling



Immunofluorescence of the glomerular capsule showing the granular deposits of immunoglobulin characteristic of nephritis. The picture is taken from the new third edition of L.A. Sternberger's *Immunocytochemistry*, published by Wiley.

is of a subjective and rather self-indulgent account of this widely used technique. Moreover, the poor reproduction of some of the plates belies the word *Atlas* in the title. Clearer images with better contrast can often be found in the original papers, and in my review copy several pages were spoiled because print was visible through the page. It is also a great pity that there are no colour plates; fluorescence microscopy is visually very appealing with the range of fluors available, an atlas of black and white plates is not.

This is no hitch-hiker's guide to the galaxy of fluorescent images obtained by immunocytochemistry. But it is welcome as the first compendium of its kind, and will be a useful work of reference for those new to the field and for teaching purposes. □

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G. A. Andres

Whitehead revealed

George Gale

Alfred North Whitehead: The Man and His Work, Vol. I 1861–1910. By Victor Lowe. *Johns Hopkins University Press*: 1985. Pp.351. \$27.50, £26.40.

Science and the Modern World. By Alfred North Whitehead. Introduction by Robert M. Young. *Free Association Books*, 26 Freegrove Road, London N7 9RQ, UK: 1985. Pp.265. Hbk £11.95; pbk £4.95.

SOME works become classics of their type. Such would seem to be the destiny of the first volume of Victor Lowe's superb *Alfred North Whitehead: The Man and His Work*. Certainly, Whitehead deserves a biography. His work as a mathematician, and as partner to Bertrand Russell in creating that original of modern mathematical logic, *Principia Mathematica*, is justification enough. But when one adds to this Whitehead's later achievements following his call in 1924 to a chair in the philosophy department of Harvard University, a biography is not just warranted, it is demanded.

Unfortunately, Whitehead himself desired that no such account of his life should be given. He systematically destroyed, or had destroyed, nearly all written traces of his personal life, including letters to and from his wife and family. Moreover, he kept no journal, believing his personal life was not a fitting subject for such a record. Finally, unlike the typical scholar, he had destroyed all of his unpublished manuscripts, drafts and various manuscript editions of his published works. Thus, he reasoned, there would be no *Nachlass* for younger scholars to waste their time searching in attempts to chronicle his intellectual development. Lowe puts all of this down to Whitehead's personal reserve: "He held an almost fanatical belief in the right to privacy, and thought that the only subject of rightful public interest in him was the work he had published" (p. 7).

Given this state of affairs, it is clear that Lowe had his work cut out. Yet his 20-year-long struggle to tease out information from impoverished sources has been well worthwhile. All of the essential ingredients of successful biography are here. Whitehead's childhood, days at school, time at Cambridge as student and fellow, marriage to Evelyn Wade in 1890, and, finally, his relationship with Russell (first as teacher, then as colleague in the founding of symbolic logic and foundations of mathematics) are each carefully laid out in all the detail that could possibly be given. Even when details are missing, and Lowe must make an inference to fill in the gap, he advises us, often most diffidently, of the fact, and then goes on with no hesita-

tion to reach a conclusion as required.

One of the more delicate of these situations concerns the beginning phases of the cooperation with Russell in 1900. Russell was apparently deeply smitten with Evelyn, and went so far as to surreptitiously support the Whitehead family to the tune of a large sum of money. Was Russell's love ever consummated? Precise details of course are lacking, yet the salient question refuses to go away. Here Lowe marshals his evidence, warns us, and makes the inference to an answer (p.248).

One major difficulty in understanding Whitehead, especially for Americans, is lack of familiarity with the English middle-class educational culture during late Victorian times. Lowe does a fine job in laying out the entire context of public schools and Oxbridge as they existed during Whitehead's era. Indeed, whether or not interest in Whitehead alone could carry this book, Lowe's account of the educational experience would deserve our attention.

Some interesting biographical points come to light. For example, Whitehead was a supreme team player, especially at rugby. His school's journal, the *Shirburnian Magazine*, called him "the best forward the School has ever had" (p.56). Lowe finds the roots of some of Whitehead's later philosophical attitudes in these experiences on the playing field. Another rich account involves the Cambridge Conversazione Society, that select, secret discussion group more commonly known as "the Apostles". Lowe provides us with a full chapter on this subject, probably about as much as could be asked for regarding a *secret* society! Several roots of Whitehead's later philosophy are to be found in his Apostolic comradeship: years spent in intense discussion with the ebullient McTaggart certainly are sufficient to account for Whitehead's later affection for idealism of the Hegelian systematic sort.

Other Apostolic tenets perhaps account for one of the severest problems that interpreters of Whitehead must face, namely, attempting to make consistent the thinking of Whitehead the English mathematician with Whitehead the American philosopher. Lowe himself eschews the project. During his exquisitely detailed analysis of the cooperative writing of *Principia*, he cautions us so:

But I shall not here make comparisons with the views to be found in what he published after he came to Harvard in 1924 as Professor of Philosophy, and must warn philosophers whose primary knowledge is of the later work that this is a very risky business [p.276].

Risky indeed, since in many ways the earlier Whitehead is manifestly inconsistent with the later. A possible explanation for this lies in the tradition of absolute candour imposed by the Apostles upon

themselves. As Sidgwick, a fellow Apostle, has noted, "No consistency was demanded with opinions previously held — truth as we saw it then and there was what we had to embrace and maintain". This attitude went deep into Whitehead's methods. Professional philosophers, Lowe observes,

like other scholars, show much concern about consistency with at least their own previous opinion. Whitehead did not. He wrote to formulate the truth as he saw it then and there, on the particular subject of his inquiry [p.115].

Evidence of this trait is not hard to find. Whitehead's most accessible, not to mention successful, philosophical treatise is his *Science and the Modern World* of 1926, which, after a ten-year hiatus, has just re-appeared in print, in an attractive edition from Free Association Books. From within the framework of a masterly historical account of the origin, rise and ultimate triumph of the modern scientific world-view, Whitehead deploys a contrary world-view, organic rather than material, concrete rather than abstract, dynamic rather than static, and laden with human values rather than free of them. Yet what is peculiar is that the most enduring aspect of this work, its superb and still-valuable critique of positivism, finds itself upon a philosophical view totally at odds with the position underlying *Principia*.

In *Principia*, the underlying metaphysical scheme is one of unconnected individuals, an atomistic pluralism. In harmony with this, the connecting relations between propositions in the axiomatic scheme consist of the weakest possible sort of implication, the so-called "material (or Russellian) implication", which abstracts completely from the meanings of the propositions, and attends "only to their being *either true or false*" (p.266).

As Lowe notes, in reference to logical atomism, "Whitehead in his mature philosophy rejected this doctrine" (p.264). Indeed, in *Science and the Modern World* Whitehead's entire critique of positivism results from his vehement attack upon Hume's philosophy, a philosophy whose notion of particular individuals and their causality are so thoroughly and well modelled by the metaphysics and logic of *Principia*.

Explanation of how this startling philosophical shift came about must await Lowe's second volume, which will begin just before Whitehead's move to America. Since this first volume will have so satisfied students of mathematics, logic, philosophy, and, indeed everyone else who has an interest in the culture of the modern world, we will all await with anticipation Lowe's account of Whitehead's transformation from English mathematician to American philosopher. □

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Recombinant vaccinia viruses as vaccines

from F. Brown, G. C. Schild and G. L. Ada

The prospect of live vaccines consisting of genetically modified vaccinia virus expressing foreign genes is exciting, but important issues concerning safety and efficacy need to be resolved.

THE World Health Organization (WHO) smallpox eradication programme, involving the extensive use of vaccinia virus vaccines, resulted in the late 1970s in the elimination of one of the world's most feared diseases. This achievement is a triumph for preventative medicine and for international collaboration in public health. The last reported case of smallpox was in 1977. In the early 1980s, WHO recommended that the routine use of smallpox vaccine should be stopped.

Against this background, the prospect of live vaccines consisting of genetically modified vaccinia virus expressing foreign antigens arising from the work of Moss¹ and Paoletti² and their colleagues has been greeted with enthusiasm. These workers have shown that genes coding for immunogenic proteins can be inserted into vaccinia virus DNA without impairing the ability of the virus to grow in tissue culture. Moreover, experimental animals infected with vaccinia virus recombinants containing genes coding for a variety of immunizing proteins have been shown to be protected against challenge infection with the corresponding infectious agent. The studies have included recombinants which express the protective antigens of hepatitis B³, rabies⁴ and malaria⁵, agents causing diseases of major economic and social importance.

The observations so far involve only small numbers of laboratory animals; the development of vaccines to a stage where they can be evaluated in the field will inevitably take time and effort. But the potential attractions of such vaccines include not only their low cost and ease of production (once suitable recombinants are established), but ease of administration and a high degree of stability, which would put them within the reach of populations in developing countries. Even so, optimism that this approach will lead to inexpensive and effective vaccines against these and other major diseases must be tempered with the concern which stems from the known adverse reactions to vaccinia virus, themselves well documented⁶ during the smallpox eradication programme.

Vaccinia virus

Vaccinia virus consists of a double-stranded DNA molecule of 187 kilobases complexed with structural polypeptides and transcriptional enzymes and enclosed

in a lipid envelope. Transcription and replication of the genome take place untypically in the cytoplasm of infected cells, which is attributable to the possession by the virus of enzymes necessary for nucleic acid metabolism. These include DNA and RNA polymerases, enzymes to cap, methylate and polyadenylate mRNA, and a thymidine kinase (TK); however, there are no RNA-splicing enzymes.

The virus DNA is non-infectious and so large that *in vitro* manipulation is difficult, for which reasons the foreign

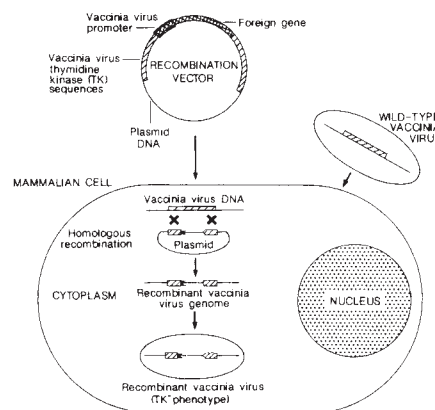


Fig. 1 Construction of vaccinia virus recombinants. The DNA sequence coding for the foreign gene is inserted into a plasmid vector downstream of a vaccinia virus promoter and flanked by vaccinia TK sequences. The resultant recombination vector is introduced into cells infected with vaccinia virus to generate a TK⁻ recombinant virus which expresses the foreign gene.

gene must be inserted into the virus by homologous recombination (Fig. 1). A plasmid vector in which the foreign gene is placed under the control of a vaccinia promoter and flanked by non-essential vaccinia virus DNA sequences is introduced into cells infected with vaccinia virus. Recombination between the flanking vaccinia sequences of the plasmid vector and the homologous sequences in the virus genome results in the insertion of the foreign gene into a specific site of the virus genome. Since, by arrangement, the insertion occurs at a position which is not essential for virus replication, the recombinant virus genome replicates normally and is packaged into infectious progeny virus.

Recombinant viruses generated in this

way are only a small proportion of the progeny, but may be distinguished from the parent virus by selective procedures: if the foreign genes are inserted within the coding sequence of the vaccinia virus TK gene this gene is inactivated and the recombinants can be selected by plaque assay on TK⁻ cells in the presence of 5-bromodeoxyuridine. This technique has now been used to select recombinants containing genes corresponding to antigens of medical and veterinary importance, influenza⁷ and herpes simplex⁸ as well as those already listed. There is little doubt that the technique is applicable to the expression of a wide range of protein or glycoprotein antigens, while several loci other than the TK gene, also inessential for virus replication, have been identified and could be valuable in constructing new recombinant viruses.

If the vaccinia virus recombinants have the same general physicochemical properties as the parent strain, they would be expected to possess the same advantages as vaccinia virus vaccines. The parent strain has a remarkable degree of stability under field conditions, a major consideration for live vaccines intended for use in tropical countries. Moreover, the vaccine can be administered easily by the dermal route, can be produced very cheaply and provides lasting immunity after a single inoculation, which is a major consideration for vaccines intended for use in developing countries. Once a suitably engineered recombinant strain of vaccinia virus has been obtained and shown to possess suitable immunogenic properties, high technology would no longer be required in vaccine production.

But before the general use of recombinant vaccinia virus vaccines is possible, several important issues concerning the safety and efficacy of vaccines of this type, will have to be resolved.

This need has been recognized by WHO, which convened two meetings, one at the US National Institutes of Health in November 1984 and a second in Geneva in September 1985. The following important conclusions were reached.

Molecular studies

Since the efficacy of the recombinants will depend on their ability to elicit a protective immunological response, further research aimed at an increased understanding of the regulation of gene expression in vac-

cinia virus will be required to ensure an adequate and stable level of expression of the foreign antigen. It will be necessary to define precisely the best ways of linking foreign genes to transcriptional regulatory sequences of the vaccinia virus genome and to identify the optimal sites for their insertion. The manner in which the foreign antigen is presented to the host's immune system should be investigated.

Level of attenuation

Vaccination with vaccinia virus can lead to abnormal skin eruptions, disorders affecting the central nervous system and a variety of other less severe complications. Thankfully, these severe complications are rare, so their occurrence with a genetically modified virus could be assessed only with a large-scale clinical study.

There is at present no generally accepted laboratory marker which predicts with certainty the virulence or attenuation of vaccinia virus or its recombinants for human beings. Reliable genetic markers of virulence would need to be established. Nevertheless, studies of mortality and pathogenesis in mice have been found to be of value; for example, the insertion of foreign genes in the TK gene has been found to reduce markedly the virulence of the virus⁹. Similar studies with other species would be valuable in telling whether this is a general phenomenon. It would be particularly useful to include primates and species such as cattle, which might be targets for immunization with recombinant vaccines. Moreover, it will be necessary, as with any attenuated virus vaccine, to study the virulence of the recombinant virus after several passages in the recipient species. Since successful immunization may require different routes of injection for different diseases, it will also be necessary to demonstrate that the recombinants are innocuous when administered by different routes.

Model systems

As there is no satisfactory laboratory animal model for assessing the virulence and efficacy of vaccinia virus, it is imperative to evaluate both *in vivo* and *in vitro* tests for their relevance in predicting the virulence for man of new recombinant strains. The results obtained should be compared with those obtained in the species for which the vaccine is intended. It will also be important to determine whether changes in host range or tissue tropism could occur as a result of the genetic modification of the virus and also by possible alterations in the viral envelope caused by insertion of a foreign gene product.

Assessing priorities

The use of vaccinia virus recombinants would be particularly advantageous for immunization against agents for which vaccines are not presently available, difficult to produce, ineffective and expensive. Moreover, the use of vaccinia virus offers the possibility of vaccination against several diseases by a single dose of a recombinant vaccine containing several gene inserts coding for different foreign antigens, but the extent to which the expression of one antigen may interfere with that of others encoded within the same genome will need to be evaluated. Studies of the immune responses of different hosts to each component antigen in multivalent recombinants will need to be carefully assessed.

In practice, it would be logical to use those vaccinia virus strains used extensively as vaccines in the WHO smallpox eradication programme and for which most information on satisfactory safety and efficacy is available. One or more well-characterized strains of vaccinia virus should be designated as references for use in the laboratory control and standardization of the vaccines.

Vaccine production

Vaccinia virus vaccine has been traditionally prepared in calf lymph, but this method of production is now considered by most authorities to be inappropriate. To be generally acceptable for use in man, it would be desirable to produce the vaccines in cell cultures employing the same good manufacturing practices as required for other modern live virus vaccines.

It is clear that control requirements for human vaccines using vaccinia virus as a vector for heterologous antigens would be developed progressively as more information relating to attenuation, production, safety and efficacy becomes available. Requirements applicable to live virus vaccines in general, such as the characteristics of the seed virus, use of a seed-lot system, description of the substrate and methods for production and the testing of safety, potency and stability, would be necessary. Issues peculiar to recombinant virus vaccines would thus include the description of the parent virus strain, methods for gene cloning, confirmation of the gene structure of the seed virus, the demonstration of its genetic stability and the results of clinical trials.

So that the results of research can be assessed for practical application as soon as possible, it is clear that WHO has an important role in encouraging and supporting the research work, in promoting

the exchange of information and establishing technical collaboration between laboratories working on the expression of protective microbial antigens by vaccinia virus recombinants. WHO also has a role in formulating guidelines and requirements for these recombinants for the standardization, control and field use of any new vaccines developed.

While most attention has been focused on the use of vaccinia virus as the vector, similar approaches using alternative vectors, including other large DNA viruses or bacterial species, are feasible and may have advantages for some applications. For example, vectors capable of replication in the gut may be more suitable than vaccinia virus in the development of vaccines against enteric infections. Moreover, vaccinia recombinants might be used for the more efficient and safer production of antigens for the development of 'inactivated' vaccines, as exemplified by the work on rabies by a group at the Wistar Institute⁴.

There is a very great potential for developing new approaches to the control of infectious disease using vaccinia virus as a vehicle, but the enthusiasm for rapid progress towards new vaccines should not be allowed to compromise the requirement for obtaining additional scientific information essential to ensure safety, efficacy and the exercise of all reasonable caution in mounting field investigations. Moreover, other approaches to vaccination are being vigorously pursued, for example controlled biosynthesis of antigens in bacterial, yeast and animal cells and site-specific mutagenesis to provide stable attenuated strains. It remains to be determined which of these approaches will provide vaccines giving optimal protection against each individual disease. □

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Horizontal shear flow in the mantle beneath the Tonga arc

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In the Tonga subduction zone, a systematic shear deformation, in which deep material moves relatively to the south, is superimposed on the mode of down-dip shortening which is characteristic of the region. We interpret this observation, together with a tectonic reconstruction, in terms of the interaction between the subducting lithospheric slab and a horizontal shear flow in the mantle.

THE Tonga arc forms the eastern margin of a complex 'vortex' of subduction (see Fig. 1) which has undergone major geometrical rearrangement in the past 20 Myr. The greatest changes are the rapid south-westward migration of the subduction west of Fiji from the Vityaz trench (AB; see Fig. 1), now inactive, to the current location at the New Hebrides arc¹⁻⁷. East of Fiji the Lau Basin has opened behind the Tonga arc in the past 4-6 Myr, generating 150-250 km of new sea floor^{5-6,8-12}. The northern termination of subduction (D) has migrated south-eastwards relative to the Pacific plate, its trace lying along a line (CD) joining Wallis Island to the current northern end of the Tonga Trench^{1,7}.

A conventional view of subduction envisages rigid slabs sinking through an essentially passive mantle. Hager and O'Connell^{13,14}, however, showed that the dips of Benioff zones can be approximately identified with the streamlines of an instantaneous global-flow model, indicating that, in fact, the geometry of subduction is strongly dependent on the pattern of flow in the mantle. Here we take the view that lithospheric material, subducted at the trench, becomes incorporated into the convective flow in the mantle; in a rapidly evolving region such as Tonga, therefore, we must interpret the current pattern of seismicity not as an instantaneous sample of an approximately steady state, but as the current locus of material particles injected into the flow by subduction in the past. The pattern of seismicity is thus a function of both the tectonic history of the region and the flow geometry of the mantle.

Assuming that the Pacific plate has behaved rigidly, the line BCD represents the original shape of the northern edge of the portion of the lithosphere subducted at the Tonga Trench. This portion of the lithosphere now forms the seismically active Benioff Zone dipping at $\sim 45^\circ$ from the Tonga Trench (DE) to the 'hook' shaped zone of intense deep seismicity (GH) south-east of Fiji.

The Louisville Ridge (EF) is a line of high sea-floor topography currently being subducted at the Tonga Trench¹⁵⁻¹⁷. The intersection of the ridge with the trench (E) is marked by a gap in shallow seismicity¹⁶⁻¹⁸. There is a corresponding gap in deep seismicity (H), which we identify with the intersection of the Louisville Ridge with the trench some 9 Myr ago¹⁹. The dipping slab DEHG represents, in this case, the current shape of the portion of the lithosphere subducted between the Louisville Ridge and the northern termination of subduction in the past 9 Myr.

The original shape of this portion of the lithosphere can be inferred by extending the line FE to intersect the Vityaz Trench at a point X between B and C. Figure 2 shows a schematic diagram of this shape, together with some approximate isochrons, indicating the time of subduction. We argue that the missing portion of the Pacific plate lying to the east of the 9 Myr isochron can be identified with the slab DEHG. The amount of distortion can be deduced by comparing the original and final

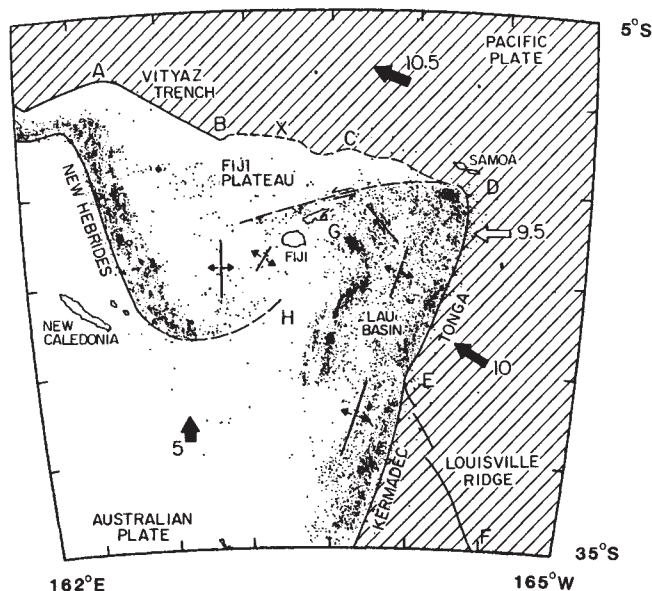


Fig. 1 Map of the south-west Pacific between long. 162°E and 165°W and lat. 35°S and 5°S. Seismicity with $m_b > 4.5$ from the ISC catalogue is shown, together with the main tectonic elements of the region. Boundaries with less-well-defined location or tectonic character are represented by dashed lines. The absolute velocities of plates are indicated by black arrows; relative velocities by white arrows. Letters indicate locations referred to in the text. 5° marks are plotted for reference on the latitude and longitude scales. The area covered by the Pacific plate in the present day is shaded.

shapes, yielding information on the velocity flow field in the mantle. The history of subduction in Tonga can be reconciled with the present-day distribution of seismicity if the zone has experienced a substantial horizontal shear deformation in which deep (~ 600 km) material has been displaced some 500 km to the south relative to surface material.

An independent observation, and, in fact, the most conclusive evidence that Tonga is undergoing such a shear deformation, is provided by the geometry of deep earthquakes in the region. Although dominated by down-dip compression¹⁹, deep earthquakes in Tonga invariably show a component of horizontal shear in which deeper material is displaced to the south.

Seismicity of Tonga Islands region

The availability of digital data has greatly facilitated the retrieval of focal mechanisms and has improved our knowledge of active processes in subduction zones²⁰. In a study focused on the deep seismicity of the Tonga Islands¹⁹ region, it was shown how the

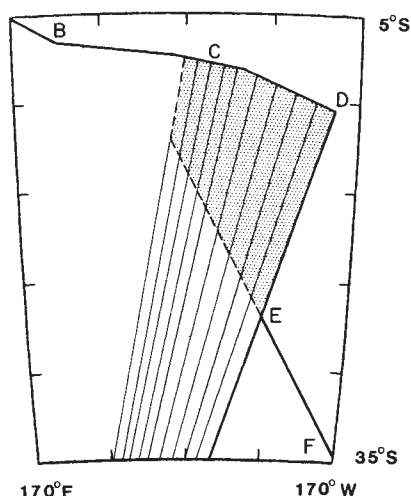


Fig. 2 Reconstruction of the Pacific lithosphere subducted at the Tonga Trench in the past 9 Myr. The tectonic elements used are depicted in Fig. 1. The geographical scale is the same as in Fig. 1. The shaded area is bounded by the Vityaz lineament (BCD) and the Tonga Trench (ED), and by the extrapolation of the Louisville Ridge (FE) along its present local azimuth of 332° and the 9-Myr isochron, obtained by using a 8 cm yr^{-1} average relative velocity and a differential opening of the Lau Basin (see text for details).

combined analysis of large numbers of reliable focal mechanisms and large sets of hypocentres reveals patterns otherwise undetected. Here we make use of the same database, hypocentres from the International Seismological Centre (ISC) catalogue for the period 1964–82 and 94 moment tensor solutions for events since 1977 deeper than 400 km, obtained with the centroid moment tensor (CMT) method^{21–27}.

Figure 1 shows the seismicity of the south-west Pacific; the prominent tectonic features of the region are shown together with the relative and absolute velocities of the plates^{7,28}. The Tonga Islands subduction zone is a region of shortening and thickening of the subducted slab, caused by the resistance encountered by the slab to penetration into the lower mantle^{19,29}. Most of the deep seismicity (and all the large events) are located on parallel shear bands, which cut across the subducted slab and are inclined at $\sim 45^\circ$ to the major compression direction (which marks the direction of the slab). The orientation of the P and N axes of moment tensor solutions is strictly connected with the geometry of these shear structures¹⁹.

In the Tonga Islands region the shear inferred from the moment tensor solutions shows a significant component in the north-south direction, perpendicular to the pattern predicted by the down-dip compression. This orientation can be seen in Fig. 3, showing a sample of typical fault plane solutions for events in the deepest part of the Tonga islands region.

The slip vectors of the solutions in Fig. 3 always have a component to the north. In the southern (events *e–j*) and northern (events *l–p*) portions of the Benioff Zone, shearing on parallel vertical bands implies motion of oceanward material relatively down, as shown by the approximately vertical fault planes (we refer to this type of focal mechanism as a type I event). It can be seen in Fig. 3, however, that the null axes of focal mechanisms on these vertical bands are systematically inclined down to the south, by an average of 18° over 36 solutions. This means that material on the oceanward side of the shear planes moves relatively down, as expected from the state of down-dip shortening, but also to the north with respect to material below ($\sim 20\%$ of the overall shear).

In the central part, instead (events *a–d*), horizontal shear is the dominant mode of deformation, taking place on subhorizontal planes dipping to the north-west with material above moving relatively north (type II events).

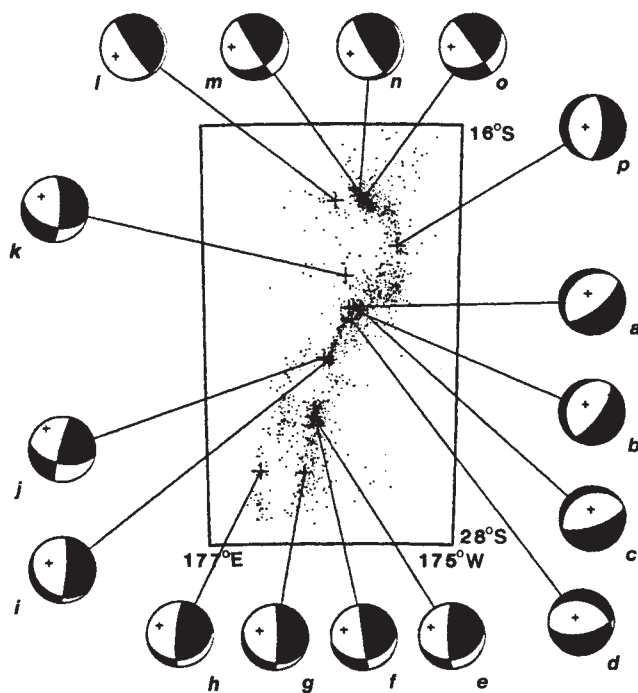
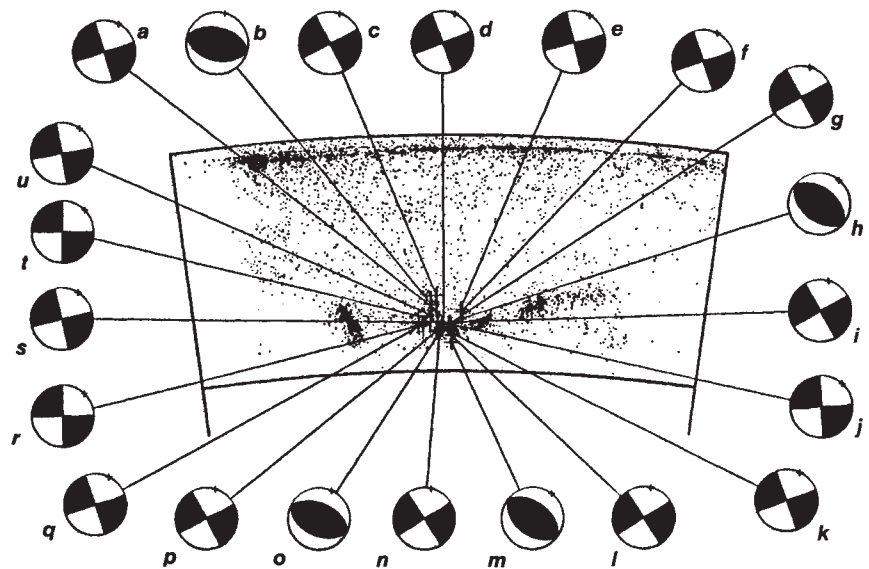


Fig. 3 Focal mechanisms for a sample of representative fault plane solutions in the Tonga Islands region. The lower hemisphere projection is shown, with the '+' indicating the direction of maximum compression. Black quadrants depict regions of extension at the source and white quadrants regions of shortening. Seismicity deeper than 400 km is shown. The events reported are: *a*, 5 July 1982; *b*, 25 November 1981; *c*, 24 April 1979; *d*, 6 February 1981; *e*, 28 April 1981; *f*, 16 September 1983; *g*, 3 November 1982; *h*, 18 April 1978; *i*, 8 July 1979; *j*, 10 April 1983; *k*, 17 June 1977; *l*, 28 January 1982; *m*, 1 January 1982; *n*, 9 November 1979; *o*, 20 July 1980; *p*, 15 May 1977.

Figure 4 shows the seismicity distribution viewed from the west, together with a representation of the moment tensors of all deep ($>400 \text{ km}$) events in the central region. Note that the 'beachballs' of this figure do not represent the complete moment tensor solution but its projection onto the vertical meridional plane. Thus, they show only that part of the moment tensor which acts to deform vertical material planes. The boundaries between black and white quadrants are the directions of maximum shear, with the sense of motion such as would take a material particle from a white into a black quadrant. Four events show a predominance of shortening, but all others show a predominance of shear deformation. Without a single exception the direction of maximum shortening, denoted by a '+', lies to the north in the lower hemisphere, indicating a net flux of material below to the south relative to material above. For events in this region we have inferred from numerous examples¹⁹ that the subhorizontal plane, dipping to the north, is usually the fault plane.

Figure 5a shows another representation of all moment tensor solutions obtained by the CMT method in the Tonga region. Each line represents the projection onto the vertical meridional plane of the axis of maximum shortening (the P-axis) of the complete moment tensor solution. The length of the line is governed by the degree of foreshortening. Again we note the strong tendency, for both deep and intermediate events, for maximum shortening to occur along lines dipping towards the north. Figure 5b shows the projections onto the vertical plane of the slip vectors of deep events, inferred by comparing moment tensor solutions with lineations in seismicity¹⁹. A bimodal distribution is indicated with clustering about directions dipping steeply (70°) and gently (20°) to the north, corresponding respectively to type I and II events defined above. Our previous study¹⁹,

Fig. 4 Seismicity of the Tonga Islands region viewed from the west, together with a representation of the moment tensors of all the events located in the central portion of the deep Benioff Zone. The projection of the complete moment tensor onto the vertical meridional plane is shown, illustrating that part of the moment tensor which acts to deform vertical meridional planes. The events reported are: *a*, 30 November 1977; *b*, 30 November 1982; *c*, 13 November 1981; *d*, 24 April 1979; *e*, 9 November 1982; *f*, 16 July 1982; *g*, 25 March 1978; *h*, 6 July 1977; *i*, 6 November 1979; *j*, 20 October 1984; *k*, 5 July 1982; *l*, 6 February 1981; *m*, 14 December 1977; *n*, 28 May 1982; *o*, 25 November 1981; *p*, 7 October 1981; *q*, 17 June 1980; *r*, 24 December 1983; *s*, 30 October 1977; *t*, 14 March 1984; *u*, 27 November 1977.



using events occurring from 1977 to 1983, found only type I events in the northern and southern regions and type II events in the central region. If continuous over a long period of time, such a shearing pattern would induce more horizontal shear in the central region of the subducted slab. Since 1984, however, the pattern seems to be reversed; type II events have been occurring in the southern region and type I events in the central region—a startling change in the pattern of activity over such a short period. Almost invariably motion is inclined to the north in the lower hemisphere.

A second striking pattern emerges from the analysis of the hypocentre distribution. We suggest that the deep seismicity is located ~500 km to the south of where it should be if the slab were subducting rigidly.

Although it is difficult to correlate a seismically-active portion of a slab with the original position on the trench at which the slab subducted, there are some cases in which this is possible; aseismic ridges, as well as corners and terminations of trenches, leave a trace in the seismicity that can be traced down the slab, sometimes to great depths^{18,30}. The two tectonic features on which we focus our attention are the Louisville Ridge and the northern termination of the Tonga Trench.

The Louisville Ridge is a pronounced aseismic chain of seamounts originating at the Eltanin fracture zone on the South Pacific Ridge¹⁵; embedded in the Pacific plate, it has been subducting at the Tonga Trench for the past few million years^{15,17}. The oblique intersection of the Louisville Ridge and the Tonga Trench marks the location of a shallow gap in seismicity¹⁶⁻¹⁸. However, attempts to locate the 'scar' left by the ridge deeper in the Benioff Zone have been unconvincing. No positive correlation with continuous gaps of seismic activity has been found on the projected location of the subducted seamount^{16-18,31}.

In a previous study¹⁹, we suggested a correlation between the prominent gap in deep seismicity located at 23°S (see Figs 1, 3) and the shallow gap located at the intersection of the Louisville Ridge with the Tonga Trench (Figs 1 and 2), on the grounds that they appear to be connected through the seismicity and because there is no known explanation for the deep gap other than its physical affinity with a similar shallow structure. The deep gap, however, is located some 500 km to the south of where the continuation of the Louisville Ridge down the slab should be^{16,18}.

Between 10 and 20 Myr ago, the Tonga Trench (ED) and the Vityaz Trench (AB) formed a continuous arc of subduction¹⁻⁷. Following the transfer of the subduction to the New Hebrides Arc, the Vityaz Trench, now embedded in the Pacific plate, has

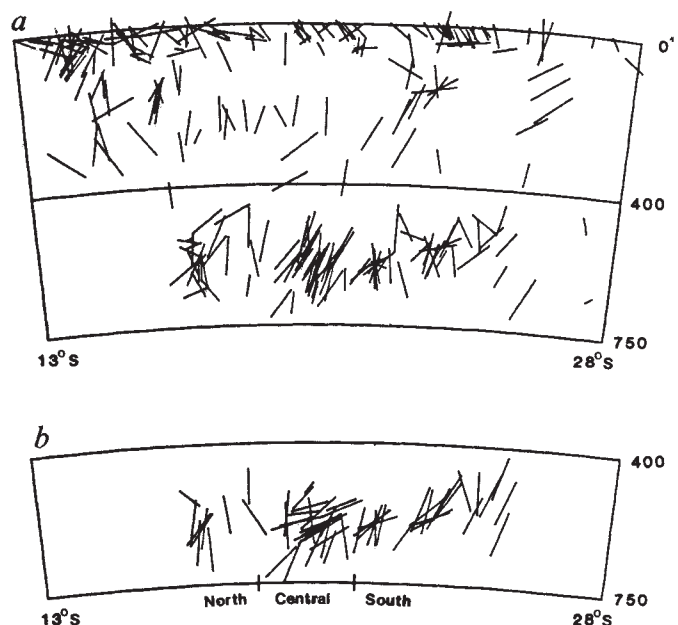


Fig. 5 *a*, All the moment tensor solutions (256) obtained with the CMT method in the Tonga Islands region are shown. Each line represents the projection onto the vertical meridional plane of the axis of maximum shortening (the P-axis) of the complete moment tensor solution. The length of each line depends on the direction of view. The 400-km depth line is shown for reference. *b*, Projection onto the vertical meridional plane of the slip vectors for all the deep events ($h > 400$ km) for which the fault plane has been identified by comparing the moment tensor solution with lineations in seismicity.

been translating to the west. The sequence of basins and troughs connecting the southern end of the Vityaz Trench with the northern limit of the Tonga Trench (BCD; hereafter referred to as the Vityaz linement) thus represents the track left by the northern termination of the Tonga Trench (D) in the Pacific plate since the Miocene. More recently, the Lau Basin began to open, displacing the Tonga Trench to the ESE by some 250 km in the past 4–6 Myr (refs 5, 8–12). The junction between the Vityaz and the Tonga trenches moved to the south-east, from its original position close to Wallis Island (C) to its present location (D)^{1,7}. This model is also supported by seismicity trends in the Lau Basin and northern Fiji Plateau³² and by detailed

petrological, seismological and geochemical analyses of the troughs forming the Vityaz linement⁷.

Based on this model, we reconstructed the shape of the lithosphere subducted at the Tonga Trench in the past 9 Myr (Fig. 2). This shape is bounded by the present location of the Tonga Trench and Vityaz linement (BCDE), the extrapolation of the Louisville Ridge (FE) along its present local azimuth of 332°, and the 9-Myr isochron based on the tectonic history of the region. The relative velocity of the Australian and Pacific plates has increased in amplitude in the past 10 Myr, from 7.1 to 9.1 cm yr⁻¹ (excluding the Lau Basin spreading rate) at the northern termination of the Tonga Trench³³, because of an increase in absolute velocity by 15% for the Australian plate^{34,35}, and 20% for the Pacific plate³⁶. The direction of the relative velocity has maintained an azimuth of 275°. To obtain the isochrons in Fig. 2 we considered an average relative velocity of 8 cm yr⁻¹ over the past 9 Myr at 15°S, and 6.5 cm yr⁻¹ at 28°S. Also, the opening of 250 km in the northern part of the Lau Basin, and 125 km in the South, is considered to have taken place in the past 5 Myr (ref. 6).

In Fig. 6a the seismicity of the Tonga Islands region is viewed from an azimuth of 266°, perpendicular to the Tonga Trench. The dashed line is the projection on a vertical plane of the shape of the Pacific lithosphere subducted at the Tonga Trench in the past 9 Myr, reconstructed in Fig. 2. The continuous line borders the area of the Benioff zone limited to the south by a line connecting the deep seismic gap with the shallow Louisville Ridge gap.

The two shapes, observed in the seismicity and predicted from the tectonic model, should correspond if no lateral deformation was active during subduction. Instead, the difference between the two is large. The seismicity seems to be shifted to the south by an amount proportional to depth, with a maximum shift of 500 km at 650-km depth.

To test the existence and the amount of the shift, we performed a simple experiment on the seismicity (Figs 6b, c). Hypocentres are shifted to the north by an amount proportional to depth, with a maximum displacement of 300 km at 650-km depth in Fig. 6b, a maximum of 500 km in Fig. 6c. Although the shift applied in Fig. 6b is not quite enough to match the seismicity with the predicted profile, the agreement between the two in Fig. 6c is remarkable, given the shortcomings of the tectonic reconstruction and the fact that we use a planar projection for a Benioff Zone which is not truly planar.

As in the case of the earthquake moment tensors, we find in the seismicity distribution another strong indication that the slab undergoes a substantial shear deformation in the north-south direction during subduction, with the deeper part of the slab moving relatively south. We estimate that a deformation of the order of 500 km in a 9-Myr period is needed to match the observed seismicity distribution.

Plate tectonics

In the case of the Tonga Islands the existence of a north-south shear flow in the mantle, required from the seismological analysis, can be explained and predicted by looking at the absolute motion of the Australian and Pacific plates.

Absolute and relative plate velocities at trenches do not usually differ appreciably, especially at oceanic-continental boundaries and in the presence of fast subducting oceanic plates²⁸. This is not always the case, however, and the Tonga Islands region provides an example of the information that can be retrieved by looking at the absolute velocities of plates.

The tectonic setting of the south-west Pacific is anomalous; the Pacific and Australian plates originate at the same ridge, and move to the north and north-west. Because the ridge is not linear and because of the large dimension of the two plates moving in a spherical geometry, the relative motion between the two plates, which move parallel south of New Zealand, becomes more and more convergent along the Melanesian border, reaching a maximum north of New Guinea, where the

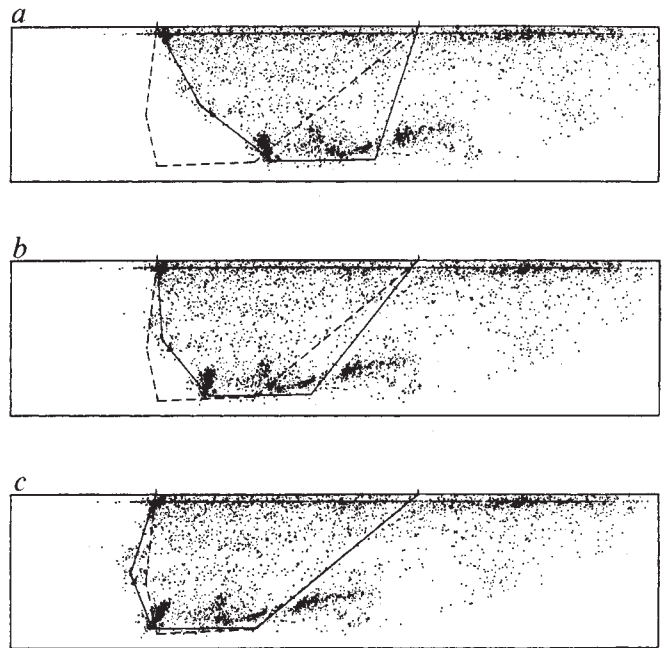


Fig. 6 *a*, Seismicity of the Tonga Islands region viewed from an azimuth of 266°, perpendicular to the Tonga Trench. The dashed line is the projection onto the vertical plane of the shape of the Pacific lithosphere subducted at the Tonga Trench in the past 9 Myr, reconstructed in Fig. 2. The solid line borders the area of the Benioff Zone limited to the south by a line connecting the deep seismic gap with the shallow Louisville Ridge gap (HE in Fig. 1). *b*, As *a*, but with the seismicity shifted to the north by an amount proportional to depth, with a 300-km maximum displacement at 600-km depth. *c*, As *b*, but with a maximum displacement of 500 km at 600-km depth.

Pacific plate is sliding towards the west and the Australian to the north, and the relative velocity reaches 13 cm yr⁻¹ (ref. 28).

At the Tonga Trench the Australian plate is moving to the north at ~5 cm yr⁻¹ (Fig. 1), in the absolute reference velocity frame in which there is no net rotation of the lithosphere (referred to as the mean mesospheric frame)^{28,33}. The Pacific plate is moving WNW³³ at 10 cm yr⁻¹ (Fig. 1). This velocity can be decomposed into components of 5 cm yr⁻¹ to the north and 8.5 cm yr⁻¹ to the west. The westerly component is responsible for the relative velocity at the trench (a smaller contribution from the back-arc spreading in the Lau Basin accounts for the trench migration). Because the relative velocity at the trench is directed east-west and the trench itself is oriented approximately north-south, the two plates, and the trench with them, are moving in the direction perpendicular to the convergence velocity at a speed comparable with the subduction speed.

This creates a unique geometrical configuration. The Pacific lithosphere is subducting in a mantle which is likely to be moving with a different velocity. For example, in the mesospheric hypothesis³³ that the mantle is stationary at 670 km, the subducted lithosphere will undergo a shear deformation of 5 cm yr⁻¹ over its entire length in the north-south direction. Assuming that the deepest part of the slab subducted ~8–10 Myr ago¹⁷, this would produce a maximum shift of some 450 km towards the south, in agreement with the estimates derived from the seismological evidence.

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Post-transcriptional regulation accounts for the *trans*-activation of the human T-lymphotropic virus type III

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The level of synthesis of viral proteins and heterologous proteins under the control of long terminal repeat sequences of human T-lymphotropic virus type III (HTLV-III or LAV) increases dramatically in cells that constitutively express the HTLV-III trans-activator protein. Increased levels of protein synthesis occur without a comparable increase in the levels of corresponding messenger RNA. We propose that post-transcriptional events mediated by the HTLV-III trans-activator protein account for positive regulation of HTLV-III gene products in infected cells.

A RETROVIRUS, the human T-lymphotropic virus type III (HTLV-III or LAV), is the primary aetiological agent of acquired immune deficiency syndrome¹⁻⁶ as well as other disorders, including a progressive degeneration of the central nervous system⁷ and lymphadenopathy^{1,2,6}. The cytopathic nature of this virus reflects the high level of virus production⁸ which can be partly attributed to virus-associated *trans*-acting factors that greatly elevate the level of gene expression directed by the HTLV-III long terminal repeat (LTR)⁹. The elements of this autostimulatory pathway include an effector, the *trans*-activator protein of relative molecular mass (M_r) 14,000 (14K)¹⁰ encoded by the bipartite *tat*-III gene^{11,12} and a responder element, the *trans*-activating responsive sequence (TAR)^{12,13}, located within the HTLV-III LTR. Elucidation of the mechanism of this autostimulatory pathway may lead to insights important in the prevention and control of virus infection.

We have used a transient gene-expression assay to examine the mechanism of *trans*-activation. Our results demonstrate that elevation of LTR-directed gene expression in cells expressing the *tat*-III protein reflects an increase in protein synthesis without a comparable rise in the steady-state level of the respective messenger RNAs. We also find that sequences that govern the *trans*-activation response must be located at or near the 5' terminus of the mRNA. We propose that positive regulation of HTLV-III gene expression mediated by the *trans*-activator protein occurs via post-transcriptional events.

Measurement of virus-encoded protein

Previous studies have shown that expression of the bacterial chloramphenicol acetyltransferase (*cat*) gene under control of the HTLV-III LTR is greatly elevated in cells that express the HTLV-III *tat* protein^{9,11-13}. An indication that positive regulation of HTLV-III LTR-directed gene expression occurs by a post-transcriptional event was our observation that the *trans*-acting responsive sequences are mainly located immediately 3' to the site of transcription initiation and comprise the 5'-terminal domain of the mRNA¹³. The unusually high levels of HTLV-III LTR-directed protein synthesis, an increase of expression of more than 500- to 1,000-fold relative to uninfected cells, also suggests that *trans*-activation of HTLV-III occurs by a mechanism other than an increase in transcription initiation. Specifically, we considered the possibility that increased protein expression is attributable to post-transcriptional events. A direct prediction of this hypothesis is that the ratio between the amount of protein and of corresponding mRNA will increase in the presence of the *trans*-activator protein.

To test our prediction we measured the relative levels of viral RNA and proteins in matched pairs of cell lines that differed only with respect to the expression of the HTLV-III *trans*-activator protein. The cell lines used for these experiments were the human lymphoid cell lines Raji (a B-cell line) and Jurkat (a T-cell line) that were uninfected or had been infected with

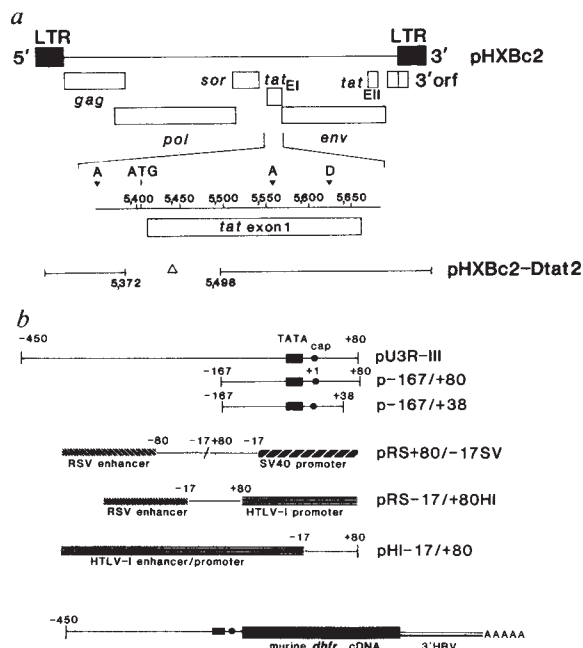


Fig. 1 Schematic diagram of plasmids used to measure *trans*-activation. *a*, Construction of plasmid pHXBc2 containing an infectious copy of HTLV-III proviral DNA has been described previously¹⁶. Plasmid pHXBc2-Dtat2 is identical to pHXBc2 except for deletion of nucleotides 5,372–5,498 in the first coding exon of the *tat*-III gene⁴⁰. orf, Open reading frame. EI and EII signify exons I and II, respectively. *b*, Plasmids pU3R-III, p-167/+80, p-167/+38, pRS+80/-17SV, pRS-17/+80HI and pHI-17/+80 contain the sequences shown directing expression of the bacterial *cat* gene²³. In each of these plasmids, polyadenylation signals obtained from the SV40 genome are present 3' to the *cat* gene. The additional plasmid sequences are derived from plasmid pBR322 (nucleotides 2,246–4,361) and provide the origin of replication and the ampicillin resistance gene. The construction of plasmid pU3R-III and p-167/+80 has been described previously^{9,13}. Plasmid p-167/+38 was constructed by deleting nucleotides +38 (*Sac* site) to +80 of the HTLV-III LTR. The RSV enhancer sequences²² present in plasmids pRS+80/-17SV and pRS-17/+80HI encompass nucleotides -138 to -229 of the RSV LTR. Both the SV40 early promoter present in plasmid pRS+80/-17SV and the HTLV-I promoter region (nucleotides -55 to +315) present in plasmid pRS-17/+80HI lack their homologous enhancer sequences. Plasmid pU3R-III-DHFR (shown at bottom) contains the cDNA⁴¹ of the murine *dhfr* gene and HBV polyadenylation sequences 3' to nucleotides -450 to +80 of the HTLV-III LTR. All plasmid constructions and DNA manipulations were done according to established procedures⁴². Solid lines depict HTLV-III LTR sequences.

a recombinant murine retrovirus *tat*-III expression vector¹⁴. These cells constitutively express a fully active HTLV-III *trans*-activator protein that stimulates HTLV-III LTR-directed gene expression. Neither the Raji (*tat*-III) nor the Jurkat (*tat*-III) cell lines express other HTLV-III-specific proteins. We have described the construction and characterization of the cell lines elsewhere¹⁴. Experiments were also performed in HeLa cells that constitutively express *tat*-III under control of the HTLV-III LTR (see Fig. 2 legend).

To determine the effect of the *trans*-activator protein on the level of HTLV-III viral mRNA and proteins, the cell lines were transfected¹⁵ with HTLV-III proviral DNA. The provirus used for this study was derived from infectious proviral DNA present on plasmid pHXBc2 (ref. 16) and contained a deletion of 94 nucleotides in the first coding exon of the *tat*-III gene (pHXBc2-Dtat2; Fig. 1*a*). The deleted provirus was used to avoid the possibility that expression of the *tat*-III gene product by the transfected provirus could complicate interpretation of the

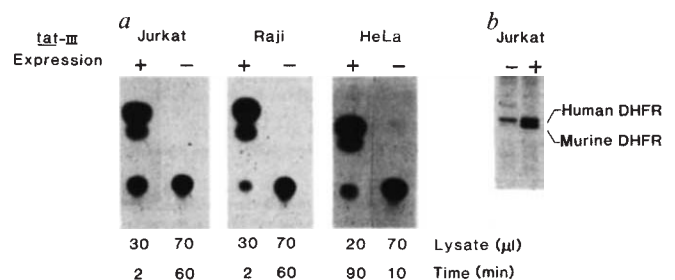


Fig. 2 Levels of HTLV-III LTR-directed CAT and DHFR protein synthesis in *tat*-III expressor cell lines. Cells were transfected with the plasmids pU3R-III (*a*) and pU3R-III-DHFR (*b*). *a*, Cell lysates were prepared 48 h post-transfection and CAT assays were performed as described previously^{23,25}. The amount of cell lysate indicated was incubated with ¹⁴C-chloramphenicol and acetyl coenzyme A for the time shown. Unreacted ¹⁴C-chloramphenicol (bottom spot) and acetylated reaction products were separated by ascending TLC²³. *b*, Cells were metabolically labelled with ³⁵S-methionine from 48 to 58 h post-transfection. ³⁵S-labelled proteins were immunoprecipitated⁴³ with anti-murine DHFR IgG and electrophoresed on 10% polyacrylamide gels. Gels were dried and fluorography was performed using Enhance (NEN). Shown are endogenous human DHFR protein (upper band) and murine DHFR protein (lower band). Cell lines that constitutively express the HTLV-III *tat* protein (+) were constructed as described previously¹⁴. The HeLa(*tat*-III) cell line was established by co-transfection of plasmids p83-5365/8053-9296 (*tat*-III expressor; ref. 12) and pSV2NEO (ref. 44), followed by selection for *neo*-resistant clones that expressed *tat*-III (ref. 44). The darker intensity of the background bands present in the *tat*-III lane probably reflects unequal loading.

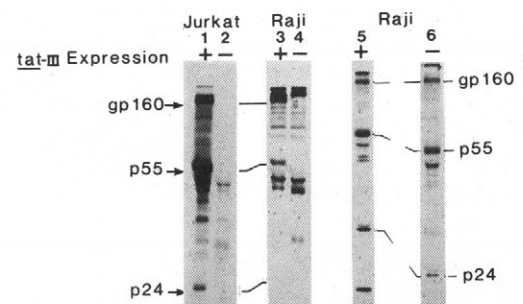


Fig. 3 Immunoprecipitation of viral proteins from transfected cells. Cell lines shown were transfected¹⁵ with plasmids pHXBc2-Dtat2 (lanes 1–4) and pHXBc2 (lanes 5, 6). Cell lysates were prepared from cells labelled 48–58 h post-transfection with ³⁵S-methionine and ³⁵S-cysteine. The virus-specific proteins were immunoprecipitated⁴³ with sera from HTLV-III-infected patients and electrophoresed on 15% polyacrylamide gels.

experiment. The deleted provirus used for this experiment was entirely defective for expression of the *trans*-activator protein as judged by its inability to increase *cat* gene expression under the control of the HTLV-III LTR in co-transfection experiments (Table 1, expt 2).

The cells used for these experiments were simultaneously transfected with two other plasmids, pU3R-III-CAT⁹, containing the *cat* gene under control of the HTLV-III LTR, and pSV2- β -globin¹⁷, containing the rabbit β -globin gene under control of the simian virus 40 (SV40) early region promoter. The increased level of CAT enzyme activity in the *tat*-III expressor cells attests to the activity of the *trans*-activator protein (Fig. 2*a*). The level of rabbit β -globin RNA in the transfected cells serves as a control of mRNA synthesized from a promoter that is not responsive to the HTLV-III *trans*-activator⁹. The SV40 early region promoter sequences present on plasmid pSV2- β -globin are identical to those present on plasmid pSV2CAT which have previously been shown to be non-responsive to the

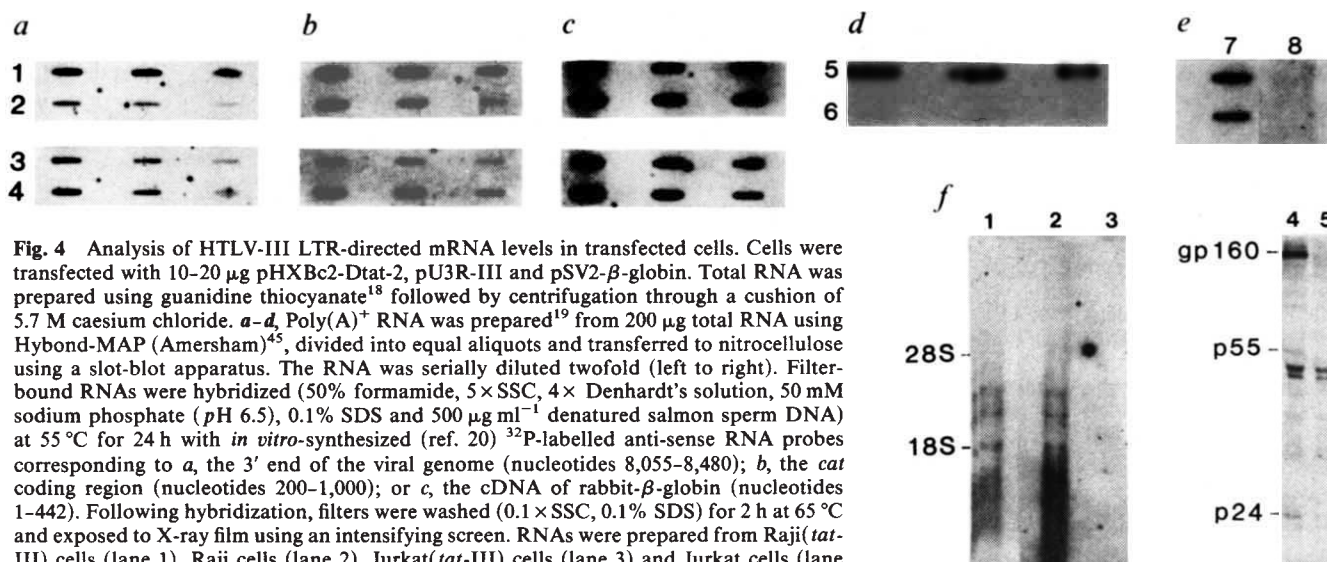


Fig. 4 Analysis of HTLV-III LTR-directed mRNA levels in transfected cells. Cells were transfected with 10–20 µg pHXBc2-Dtat-2, pU3R-III and pSV2- β -globin. Total RNA was prepared using guanidine thiocyanate¹⁸ followed by centrifugation through a cushion of 5.7 M caesium chloride. **a–d**, Poly(A)⁺ RNA was prepared¹⁹ from 200 µg total RNA using Hybond-MAP (Amersham)⁴⁵, divided into equal aliquots and transferred to nitrocellulose using a slot-blot apparatus. The RNA was serially diluted twofold (left to right). Filter-bound RNAs were hybridized (50% formamide, 5 $\times$ SSC, 4 $\times$ Denhardt's solution, 50 mM sodium phosphate (pH 6.5), 0.1% SDS and 500 µg ml⁻¹ denatured salmon sperm DNA) at 55 °C for 24 h with *in vitro*-synthesized (ref. 20) ³²P-labelled anti-sense RNA probes corresponding to **a**, the 3' end of the viral genome (nucleotides 8,055–8,480); **b**, the *cat* coding region (nucleotides 200–1,000); or **c**, the cDNA of rabbit- β -globin (nucleotides 1–442). Following hybridization, filters were washed (0.1 $\times$ SSC, 0.1% SDS) for 2 h at 65 °C and exposed to X-ray film using an intensifying screen. RNAs were prepared from Raji (*tat*-III) cells (lane 1), Raji cells (lane 2), Jurkat (*tat*-III) cells (lane 3) and Jurkat cells (lane 4). **d**, poly(A)⁺ RNA was prepared from HeLa (*tat*-III) cells transfected with plasmid pHXBc2-Dtat2 (lane 5) and mock-transfected HeLa *tat*-III cells (lane 6) and probed with anti-sense RNA corresponding to the *cat* coding region. **e**, Total RNA, prepared from HeLa (*tat*-III) cells transfected with plasmid pU3R-III, was treated with DNase (lanes 7, 8) and RNase (lane 8), transferred to nitrocellulose then hybridized with anti-sense *cat* RNA. **f**, Northern blot analysis and protein analysis of virus-specific mRNA and protein. Total RNA was isolated from transfected cells as described above. RNA (20 µg) obtained from pHXBc2-transfected Raji cells (lane 1), pHXBc2-Dtat2-transfected Raji cells (lane 2) and Raji cells transfected with HTLV-I proviral DNA (plasmid pXT1env contains the HTLV-I LTR directing expression of the HTLV-I *env* gene) (lane 3) was electrophoresed through 1% formaldehyde agarose gels and transferred to nitrocellulose filters²¹. Filter-bound RNA was hybridized for 24 h (50% formamide, 5 $\times$ SSC, 4 $\times$ Denhardt's solution, 50 mM sodium phosphate (pH 6.5), 0.1% SDS and 500 µg ml⁻¹ denatured salmon sperm DNA) at 55 °C to a ³²P-labelled DNA probe spanning the complete genome. Filters were washed at high stringency (65 °C; 0.2 $\times$ SSC, 0.1% SDS) and exposed to X-ray film and mRNA species sized using methylene blue-stained ribosomal RNA as size markers. Lanes 4 and 5 show virus-specific proteins present in the HXBc2 and HXBc2-Dtat2-transfected cells, respectively. Immunoprecipitations and protein analysis were performed as described in Fig. 3 legend.

tat-III product^{9,12,13}. In a separate experiment the same cell lines were simultaneously transfected with plasmid pSV2- β -globin and plasmid pU3R-III-DHFR (Fig. 2b), which contains the complementary DNA of murine dihydrofolate reductase (*dhfr*) under control of the HTLV-III LTR. Figure 2b demonstrates that synthesis of the murine DHFR protein was greatly elevated in the *tat*-III expressor cells. The level of murine *dhfr* mRNA could not be quantitated because of co-migration on Northern gels with endogenous human *dhfr* mRNA.

The level of viral protein expression was determined by incubation of the pHXBc2-Dtat2 transfected cells with ³⁵S-methionine and ³⁵S-cysteine for 10 h, 48 h post-transfection. Virus-specific proteins were detected by immunoprecipitation with antisera from HTLV-III-infected patients followed by analysis on polyacrylamide gels. These experiments demonstrate that expression of virus-specific proteins is greatly increased in Raji (*tat*-III) compared with Raji cells (Fig. 3). Identical results were obtained on transfection of Jurkat and Jurkat (*tat*-III)-infected cells (Fig. 3). The extent of stimulation of virus-specific protein is >500-fold as determined by densitometric comparison with a host protein present in uninfected cells. No HTLV-III-specific proteins were detected upon transfection of uninfected Raji and Jurkat cells with the deleted provirus. Note that the well-characterized viral proteins gp160, p55 and p24–25 are evident in analysis of immunoprecipitates from the transfected cells. In a control experiment, pHXBc2, containing an intact infectious provirus, was transfected into uninfected Raji cells and Raji (*tat*-III) cells. Figure 3 demonstrates that the same virus-specific proteins were evident following transfection of both cell lines.

Measurement of mRNA

The level of virus-specific mRNA was determined by isolation of total cellular RNA with guanidine thiocyanate¹⁸, centrifugation through caesium chloride and selection of poly(A)-containing RNA species¹⁹. The poly(A)⁺ fraction was transferred to nitrocellulose filters and the concentration of virus-

specific RNA was determined using *in vitro*-synthesized²⁰ ³²P-labelled anti-sense viral RNA corresponding to the 3' portion of the provirus, a region of the genome not present in the retroviral vector used to introduce the HTLV-III *trans*-activator gene into the lymphoid cell lines. The steady-state level of *cat* mRNA and rabbit β -globin mRNA was also measured in these experiments using the respective ³²P-labelled probes. RNA and protein analysis were done using the same cell preparations at the same time.

Our results show that the level of viral mRNA relative to β -globin mRNA is similar both in uninfected cells and in cells that express the HTLV-III *trans*-activator protein (Fig. 4). The level of *cat* mRNA, also under control of the HTLV-III LTR, remains constant relative to the level of β -globin mRNA that is initiated in the SV40 promoter (Fig. 4). RNA was also prepared from untransfected cells. Figure 4d demonstrates that the hybridization signals are well above the background level. Digestion of the samples with RNase also eliminated the signal.

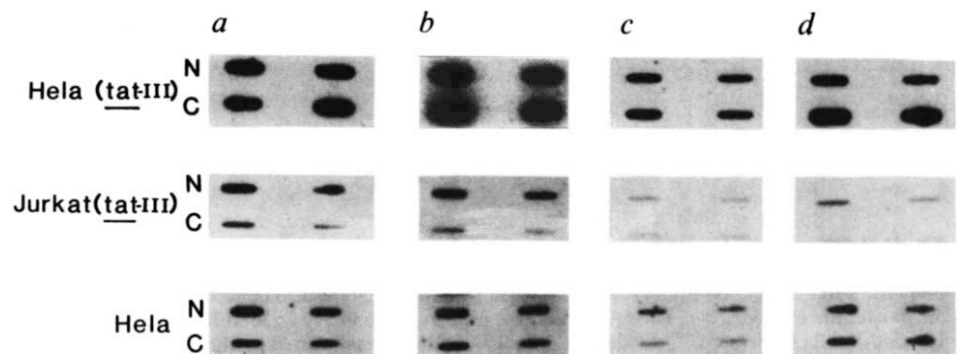
Intracellular distribution of viral mRNA

We compared the level and the length of viral mRNA species in the transfected cells by Northern blot analysis²¹. Figure 4f shows that similar amounts of viral mRNA species are present in Raji cells transfected with plasmid pHXBc2-Dtat2 and HXBc2. No signal was observed in control cells transfected with HTLV-I proviral DNA present on plasmid pXT1env (see Fig. 4f legend). Virus-specific proteins were only evident in those cells transfected with plasmid HXBc2 (Fig. 4f), which expresses the *tat*-III product. The proteins were extracted from ³⁵S-methionine- and ³⁵S-cysteine-labelled cells at the same time post-transfection as the RNA was extracted. The low level of viral RNA observed (Fig. 4f) is typical of measurements of RNA done under transient transfection conditions using cell lines that do not incorporate large amounts of DNA.

The intracellular distribution of viral RNA was determined by fractionation of the cells into nuclear and cytoplasmic components. The HeLa (*tat*-III) and Jurkat (*tat*-III) cells used for

Fig. 5 Subcellular distribution of mRNA in *tat*-III expressor cells. HeLa(*tat*-III) cells, HeLa cells and Jurkat (*tat*-III) cells were transfected with pU3R-III, pSV2- β -globin and pHXBc2-Dtat2. At 48 h post-transfection, cells were lysed using the non-ionic detergent NP40 (ref. 42) and nuclear and cytoplasmic fractions were isolated, then extracted with guanidine thiocyanate. RNA was pelleted through a cushion of caesium chloride, poly(A)⁺ RNA was prepared^{19,45}, diluted twofold and transferred to nitrocellulose.

Hybridization and washing conditions were as described in Fig. 4 legend. *In vitro*-synthesized anti-sense RNA probes were prepared from DNA corresponding to: *a*, the 3' end of the viral genome; *b*, the *cat* gene; *c*, rabbit β -globin cDNA; *d*, the murine *dhfr* cDNA (nucleotides 1-1,115). Nuclear (N) and cytoplasmic (C) RNAs are indicated.



this study were simultaneously transfected with pHXBc2-Dtat-2, pU3R-III and pSV2- β -globin. The concentration of viral RNA, SV40-directed β -globin RNA and RNA synthesized from the cellular *dhfr* gene was measured in each fraction. Figure 5 demonstrates that the presence of the *trans*-activator protein did not affect the relative distribution of viral RNA or HTLV-III LTR-directed *cat* mRNA in the nucleus and cytoplasm in either the Jurkat(*tat*-III) or HeLa(*tat*-III) cells. The distribution of mRNAs was similar to that of the β -globin RNA synthesized from the SV40 promoter or to the endogenous *dhfr* mRNA. Similar results were obtained in parallel experiments performed with the control cells that lack *tat*-III expression (Fig. 5).

We conclude that the greatly elevated levels of virus protein and of CAT enzyme activity detected in cells that express the HTLV-III *trans*-activator protein occur without a comparable increase in the level of the corresponding mRNA and do not result from an alteration in the ratio of nuclear to cytoplasmic mRNA.

Position of TAR sequences

In a previous study, deletion analysis of the HTLV-III LTR demonstrates that the sequence necessary for *trans*-activation (the TAR sequence) is located between nucleotides -17 and +80 (initiation of viral RNA occurs at +1) (ref. 13). We next investigated the requirement for *trans*-activation of the position of the TAR sequences relative to the site of transcriptional initiation. Plasmids were constructed in which the TAR element was placed either 5' to the site of RNA initiation, or alternatively inserted near, but not at, the 5' end of the mRNA species (Fig. 1b). For these experiments the -17 to +80 HTLV-III sequences were inserted between an enhancer sequence derived from the Rous sarcoma virus (RSV)²² and one of two promoter sequences, the SV40 promoter²³ or the HTLV-I promoter²⁴, each lacking the homologous enhancer. These control sequences were placed 5' to the *cat* gene. The ability of each of these plasmids to direct *cat* gene expression in a transient expression assay was determined by transfection of either the Raji or Raji(*tat*-III) cells.

TAR sequences do not confer a responsive phenotype when placed 5' to the heterologous promoters in these plasmids (Table 1). The lack of response of the control elements is in marked contrast to the response of heterologous promoters that contain the TAR sequences located 3' to the site of initiation¹³ (Table 1). The sequences located between +38 and +80 are required for *trans*-activation (Table 1) (compare the activity of plasmid p-167/+38 to that of p-167/+80).

The structure of the second type of plasmid (pHI-17/+80) is also shown in Fig. 1b. Here, the TAR sequences are located 3' to the HTLV-I enhancer/promoter, 300 nucleotides from the natural site of initiation of the HTLV-I viral RNA. RNA transcribed from this plasmid initiates within the HTLV-I LTR sequences at a position ~300 nucleotides 5' to the HTLV-III

start site (not shown). The data in Table 1 show that the level of *cat* gene expression directed by plasmid pHI-17/+80 is not significantly different in Raji compared with Raji(*tat*-III) cells.

We conclude that *trans*-activation of HTLV-III requires that the TAR sequences are located at or very near the 5' end of the mRNA. The experiments also show that, consistent with earlier results, the sequences located within the untranslated leader region (+38 to +80) are required for *trans*-activation of homologous and heterologous genes.

Discussion

The strategy of replication of HTLV-III includes a positive feedback mechanism in which one product of the virus genome, the *tat*-III protein, positively regulates expression of other viral proteins. Experiments presented here suggest that positive regulation of HTLV-III genes occurs via an increased translational efficiency of virus-specific mRNA species. Our experiments also demonstrate that the increased translational efficiency requires specific sequences within the R region of the LTR, the TAR sequences, to be located at or near the 5' terminus of the mRNA.

The simplest explanation of these data is that the *tat*-III gene product itself interacts with sequences at the 5' terminus of mRNA species and thereby increases the translational efficiency of that mRNA. However, the experiments reported here do not exclude other mechanisms of post-transcriptional control. The

Table 1 Activity of HTLV-III LTR sequences in Raji and Raji(*tat*-III) cells and co-transfection assays

Expt	Plasmid	Relative CAT activity		
		Raji	Raji(<i>tat</i> -III)	HeLa
1	pSV2CAT	1.0	1.0	
	pU3R-III	0.3	241 (777)	1.0
	p-167/+80	0.7	1,363 (1,948)	
	p-167/+38	0.8	1.7 (2)	
	pRS+80/-17SV	0.2	0.3 (1.5)	
	pRS-17/+80HI	0.8	0.6 (0.75)	
	pHI-17/+80	1.2	1.2 (1.0)	
2	pU3R-III+HXBc2			26.0
	pU3R-III+HXBc2-Dtat2			1.2

In expt 1, cells were transfected with 2 μ g of the plasmid DNA indicated and CAT assays were performed 48 h post-transfection^{23,25}. The level of CAT enzyme activity was normalized to the level obtained following transfection of the same cells with pSV2CAT. The SV40 sequences directing *cat* gene expression on pSV2CAT are not responsive to the *tat*-III gene product⁹. The numbers in parentheses represent the fold stimulation in Raji (*tat*-III) compared with Raji cells. In expt 2 pU3R-III alone was transfected into HeLa cells or co-transfected with 2 μ g of either pHXBc2 or pHXBc2-Dtat2. The level of CAT activity was normalized to the activity obtained following transfection with pU3R-III alone.

14K *trans*-activator protein contains a cluster of positively charged amino acids, suggesting that it interacts directly with viral RNA¹⁰. Alternatively, but in our opinion less likely, a cellular product may be induced by the *tat*-III gene which affects the translational efficiency of viral RNA.

We previously reported higher levels of LTR-directed mRNA in HTLV-III-infected H9 cells compared with uninfected cells¹³. The absolute increase could not be determined because the steady-state level of the corresponding mRNA in the uninfected cells was not measurable. The increased level of mRNA could reflect either transfection differences between the cells or the presence of additional factors in HTLV-III-infected cells that could stabilize the mRNA. Because the data presented here were derived from matched cell lines that differ only with respect to expression of *tat*-III, we believe that these results reflect more accurately the effect of *tat*-III expression on virus gene expression. Moreover, the increase in mRNA observed in some experiments reported here cannot explain the greatly elevated levels of HTLV-III LTR-directed *cat* gene expression and viral protein synthesis in cells expressing *tat*-III.

Stimulation of gene expression by a viral protein has been observed for gene expression directed by LTR sequences of HTLV-I and HTLV-II²⁵⁻²⁸ and of the bovine leukaemia virus (BLV)^{29,30}. The *trans*-activator protein for HTLV-I and -II is encoded near the 3' end of the genomes, by the *tat*-I and *tat*-II genes, respectively^{27,31}. Although the ultimate effect of the *trans*-activator proteins of HTLV-I, HTLV-II, BLV and HTLV-III is similar, it is likely that the mechanism of *trans*-activation differs. One indication of this difference is the disparity in sizes of the *trans*-activator proteins. The *tat*-III protein is 86 amino acids long, whereas the *tat*-I and *tat*-II proteins are >300 amino acids long. Another difference is the location of the *trans*-acting responsive sequences. The TAR-I and TAR-II sequences of HTLV-I and -II are located within the U3 regions of the respective LTRs, 5' to the site of transcription initiation^{24,32}. Finally, there is experimental evidence which demonstrates that at least part of the increase in LTR-directed gene expression in response to these *trans*-activator proteins occurs via an increased rate of transcriptional initiation and concomitant increases in the steady-state levels of mRNA species^{25,27}. Evidently, different biochemical mechanisms account for *trans*-activation of gene expression mediated by the *tat*-I and *tat*-III genes of HTLV-I and HTLV-III, respectively.

Genetic regulation by alteration of the translational efficiency of mRNA has been reported for some cellular and viral genes. For example, a sequence present within the 5'-untranslated

leader of the *Drosophila melanogaster* heat-shock protein (HSP70) mRNA is required for continued translation of this mRNA under conditions of stress^{33,34}. Tagging of heterologous genes with the HSP70 leader sequence transfers the heat-shock translation phenotype to heterologous genes such as tagging heterologous genes with the TAR sequences of HTLV-III confers responsiveness to the type III *trans*-activator. Moreover, deletion of nucleotides within the HSP70 untranslated leader and insertion of heterologous sequences 5' to this region abolishes selective translation³⁴. Deletions within the HTLV-III leader sequence and insertions 5' to the HTLV-III TAR sequences have a similar effect. A sequence in the untranslated leader of viral mRNAs expressed late in adenovirus infection permits continued translation of these mRNAs at times when translation of most other viral and cellular genes stops^{35,36}. Translation of picornavirus mRNAs also continues at a time when translation of host genes is diminished^{37,38}.

Although some aspects of translational regulation are common among these disparate examples, note that high levels of protein expression mediated by the *tat*-III gene product occur in cells that retain the capacity to synthesize other proteins. Viewed from this perspective, translational control mediated by the HTLV-III *tat* gene product could serve as a prototype for a distinct category of genetic regulatory events. Perhaps similar mechanisms are involved in translational activation of mRNA in developing zygotes and the activation of specific genes in response to extracellular stimuli and to stress in situations in which large amounts of protein are made quickly³⁹.

We have demonstrated that the *tat*-III product is essential for virus replication in T4+ cell lines⁴⁰. For this reason, inhibitors of HTLV-III *trans*-activation should inhibit viral replication and may moderate the effects of virus infection. The observations reported here suggest that drugs that interfere with post-transcriptional events, possibly certain antibiotics which inhibit translational processes, may specifically inhibit virus growth.

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Three-dimensional structure of a specific pre-messenger RNP particle established by electron microscope tomography

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Electron microscope tomography has been used to refine the structural analysis of individual RNP particles synthesized on Balbiani ring genes in Chironomus tentans. The spherical particles have a diameter of 500 Å and are composed of a thick RNP ribbon bent into an asymmetrical, four-domain, ring-like configuration. The first domain, containing the 5' end of the transcript, and the fourth domain, containing the 3' end, are close to each other.

IN eukaryotic cells messenger RNA precursors, the heterogeneous nuclear RNA (hnRNA) transcripts, are packed into ribonucleoprotein particles (hnRNP) immediately on synthesis^{1,2}. The particles exhibit a monotonous polyparticle organization³ and contain a set of 5-10 major protein components, the core proteins^{2,4-7}. However, various lines of evidence suggest that each transcription product has characteristic features. For example, defined RNP particles display a specific organization of the proteins along the RNP fibre⁸⁻¹⁰; some of the minor protein components of hnRNP bind to specific RNA sequences¹¹, whereas others are confined to certain transcription products¹²⁻¹⁴. At the ultrastructural level, the hnRNP particles at defined loci in polytene¹⁵ and lampbrush¹⁶ chromosomes show typical morphology. Because of the apparent complexity of the hnRNP population, it is important to study defined hnRNP particles and to relate their structure to the maturation processes that occur on the particles¹⁷.

Electron microscope studies of selected chromosomal regions of insect polytene chromosomes permit analysis of specific hnRNP particles. In particular, Balbiani rings (BRs, the giant puffs in the salivary glands of *Chironomus*) have proven useful (see ref. 18 for review). The Balbiani ring (BR) genes encode information for the salivary polypeptides, the major protein products of the salivary gland cells. Large 75S RNA transcripts are synthesized in the BRs¹⁹ and packed into 500 Å RNP particles²⁰⁻²³. The BR particles can be readily identified in the nuclear sap and in the nuclear pores during the translocation process^{24,25}. Here we describe a general method for three-dimensional reconstruction of ultrastructural objects at the molecular level. By applying this method to the BR particles we could establish whether they attain a well-defined, higher order structure. We discuss their structure in relation to the available information on the transport, polyadenylation and splicing of the BR transcripts.

Image reconstruction

Ultrathin sections through the salivary glands of *C. tentans* were prepared according to standard procedures²¹ and gold particles deposited onto the surface of the sections. A suitable nuclear area containing BR granules was identified in the electron microscope (Fig. 1), a tilt series recorded and the micrographs scanned and digitized.

Before the reconstruction was performed, the micrographs were aligned with an iterative least-squares procedure (G. Bricogne and U.S., in preparation). The gold particles on top of the section served as reference markers.

The three-dimensional reconstruction was performed according to the filtered back-projection principle where the Fourier transforms of the projections (tilts) are radially weighted²⁶. The reconstruction comprised a series of two-dimensional recon-

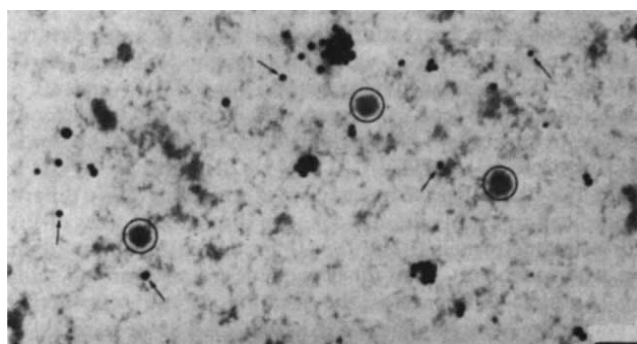


Fig. 1 Electron micrograph of a nuclear region of a salivary gland cell in *C. tentans*. Three differently orientated BR RNP particles are indicated by circles. The particles appear associated with a fibrous network. Arrows denote some of the gold particles added to the specimen. This micrograph represents 0° tilt in a $\pm 60^\circ$ series of 13 micrographs. Scale bar, 1,000 Å.

Methods. Specimen preparation, electron microscopy and image processing were performed as follows. Salivary glands from fourth instar *C. tentans* larvae were fixed in 2% glutaraldehyde and post-fixed in 1% osmium tetroxide. After embedding and sectioning, the specimens were stained with uranyl acetate and lead citrate (see ref. 21 for details). Thin sections were collected directly onto 200-mesh copper grids without a supporting plastic film. A colloidal gold suspension (Polysciences) was added to the sections, and gold particles (50-200 Å in diameter) deposited on the top. The specimens were photographed at 60 kV at a magnification of 20,000 $\times$ in a JEOL TEMSCAN 100 CX electron microscope and a tilt series obtained using a standard side-entry goniometer. Micrographs were scanned and digitized in an Optronix P-1000 drum scanner. The scanning was performed with a step size of 25 μ m, which corresponds to a pixel size of 12.5 Å at a magnification of $\sim 20,000\times$. For each micrograph, an area $\sim 50\text{ mm} \times 50\text{ mm}$ was scanned, that is, 4×10^6 pixels were stored. Subsequent image processing was carried out on a VAX 11/750 minicomputer.

structions of circular disks perpendicular to, and repeated along, the tilt axis, forming a cylinder (Fig. 2). The completed reconstruction was presented as a series of stain density maps corresponding to the reconstructed sections through the object. The maps were subsequently used to build a balsa-wood model of the object.

Evaluation of the method

Our three-dimensional reconstruction technique is based on that of Klug and coworkers, devised for symmetrical and repeating ultrastructural objects^{26,27}. Here, the reconstruction is made from a series of projections that correspond to the micrographs of

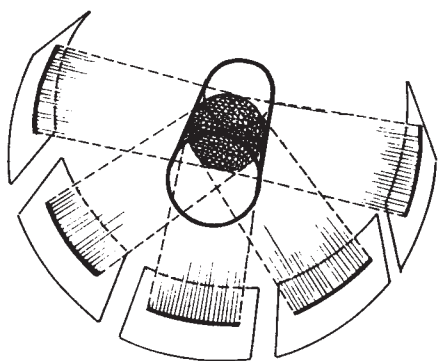


Fig. 2 The principle of three-dimensional image reconstruction from electron micrographs. Five micrographs, shown in a semicircular arrangement, represent a set of projections of a centrally placed specimen which are accurately aligned in relation to each other to allow image reconstruction. The three-dimensional reconstruction takes place as a series of two-dimensional reconstructions: in each single step, one of which is shown in the figure, a circular disk is reconstructed by back-projection (after suitable weighting) of the corresponding lines in the micrographs^{47,48}. Note that the lines and the reconstructed disk are perpendicular to the tilt axis. Several consecutive disks ultimately form a cylinder in which the reconstructed object is contained.

differently tilted views of the specimen, in which any object, symmetrical or asymmetrical, can be analysed (electron microscope tomography). The feasibility of the approach has been shown previously^{28,29}. The key to the success of the tomographic procedure is the proper alignment of the electron micrographs in the tilt series—the data retrieved from each micrograph must correspond to the particular object orientated in the same way (except for a modulation with the tilt angle). The use of gold markers as reference markers³⁰ and the development of accurate algorithms for the alignment have allowed us to restrict the average alignment errors for the gold markers between the 0° and higher tilts to ~10 Å (corresponds to <1 pixel). Care was also taken to minimize resolution loss caused by multiple resampling of the data in the computer.

The resolution of the tomographic procedure could be estimated from the reconstructed density maps computed at different resolutions. The characteristics of the maps suggested a resolution of 80–90 Å, which approaches the maximum attainable resolution calculated to be ~85 Å for a 500-Å object under optimal conditions (no background; projections available for every 10° in the range ±90°)²⁶. Moreover, computation of an average structure from several reconstructions of individual but identical objects further increases the reliability of the results obtained. Preliminary results in our laboratory show that a resolution <50 Å can be reached when the number of projections are doubled. We conclude that our method is of general applicability in the analysis of objects of molecular dimensions.

Structure of individual BR particles

Three BR granules are shown in Fig. 1. The RNP particles of BR origin are of exceptional size (~500 Å) and appear roughly spherical, but their contour indicates that they are often not homogeneous. Furthermore, they frequently exhibit a bright centre, giving a ring or horseshoe appearance (see, for example, the RNP particle to the right in Fig. 1).

Information was obtained previously by direct visualization of the synthesis of the gene product²³. It was shown that the RNP particle is formed as a thick fibre that is bent into an almost closed structure, providing a tentative explanation for the different projections observed for the completed particles. Although this study using conventional electron microscopy provided an approximate interpretation of the structure of the RNP particle, we were technically unable to describe completely the complex, spherical appearance of the particle, as neither

the course of the RNP fibre nor its shape could be established. Using computer-based electron microscope tomography, we have now performed three-dimensional reconstructions of four BR particles from glands of different animals (Fig. 3a–d).

The degree of similarity between the reconstructions of the four particles was determined by a least-squares procedure that maximizes the correlation coefficients between the reconstructed densities by refining relative three-dimensional translation and rotation parameters^{31,32}. When the particle in Fig. 3a was compared with each one of the other three particles (b–d), we obtained well-defined, maximal correlation coefficients of 0.75, 0.82 and 0.77, respectively, indicating a strong similarity between the four particles. In this type of comparison all the information in the reconstructions is considered, whereas the balsa-wood models (Fig. 3) only allow the most heavily stained features to be evaluated at the chosen contour levels (see Fig. 3 legend).

The BR particles are globular in appearance and have a diameter of ~500 Å (Fig. 3). They are asymmetrical and the contours of the particles in the various projections are similar; for example, in Fig. 3 the aligned particles exhibit a pointed bottom and a rhomboid contour. Furthermore, the particles have a central hole (with a few crossing fibres; see Fig. 3 legend) and also contain a slit at 1–2 o'clock cutting through the periphery of the particle (the position of the slit is indicated by an arrow in Fig. 3). The presence of the cleft is compatible with the horseshoe-like projections observed earlier; it is assumed that the ends of the RNP element are in proximity with each other at the cleft. Thus, we are left with a toroidal structure cut open with a narrow slit. The reconstructions indicate that the ring-shaped element is better described as a ribbon than as a fibre as it is considerably wider than it is thick.

When minor features are scrutinized, it is possible to detect differences between the particles (Fig. 3). Some of the variation can be attributed to differences in stain distribution, particularly as the balsa-wood models only represent the stain densities exceeding the selected cut-off level. Biological variation could also contribute to the differences observed; that is, the particles could be of different BR origin (BR 1 or BR 2)¹⁸, one or more of them could be processed or partly degraded, or they could represent different dynamic states of the particle. Note, however, that at the resolution of 80–90 Å there is a striking resemblance between the four particles.

Average structure

The average structure was established by adding together the four reconstructions corrected for translation, rotation and scaling factors. *R*-values were computed as in conventional X-ray crystallography. When the individual particles (Fig. 3a–d) were related to the average, we obtained *R*-values of 0.44, 0.50, 0.41 and 0.46, respectively. These reasonably low values, as well as the high correlation numbers presented in the preceding section, provide evidence that the four BR RNP particles have the same structure at this resolution and can be represented by the computed average. The average structure is presented as a balsa-wood model in Fig. 3e; the toroid shape of the particle as well as the slit are clearly visible.

A more detailed description of the RNP element can be carried out with close reference to Fig. 4a–f, which displays the average particle from several views. Most conveniently, the RNP element is described by beginning at the slit, following it clockwise around the particle and finishing at the slit again. First, the RNP element, constituting the wall of the particle, has about the same thickness all around the particle (100–150 Å) and moulds itself into the general spherical shape of the particle. Second, when the particle is observed from the outside along the RNP element, it is possible to demarcate four domains, designated 1–4 (see Fig. 4a, b). Domain 1 starts as a smooth end at the slit at 1 o'clock and attains a width of ~300 Å and domain 2 begins at 3 o'clock when the RNP element rather abruptly broadens to 500–600 Å, reaches the bottom of the particle (Fig. 4d), makes

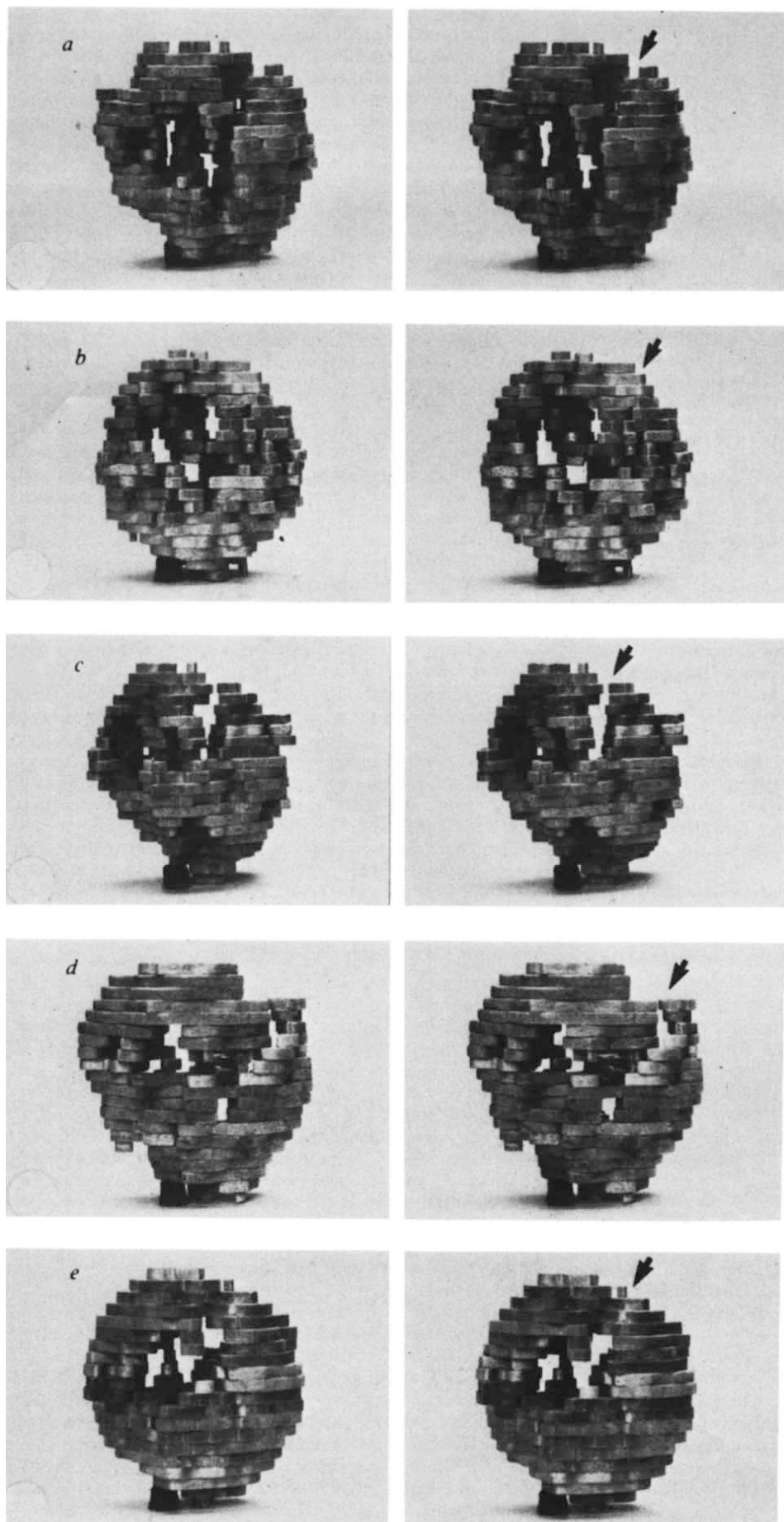


Fig. 3 Balsa-wood models of four reconstructed and aligned BR RNP particles (*a-d*) and a calculated average particle (*e*) shown as stereo pairs. The balsa-wood models of the four individual particles were built from stain density maps contoured at ~ 2.4 r.m.s. deviations above the mean value of the reconstructions, whereas the computed average of the four reconstructions was contoured at 1.15 r.m.s. Short arrows denote the position of the slit in the toroid-like structure. Nuclear sap fibres next to the particles have not been included in the models except for those inside the cavity of the particles. The reconstructions are compensated for a 10% reduction in thickness of the specimen during exposure.

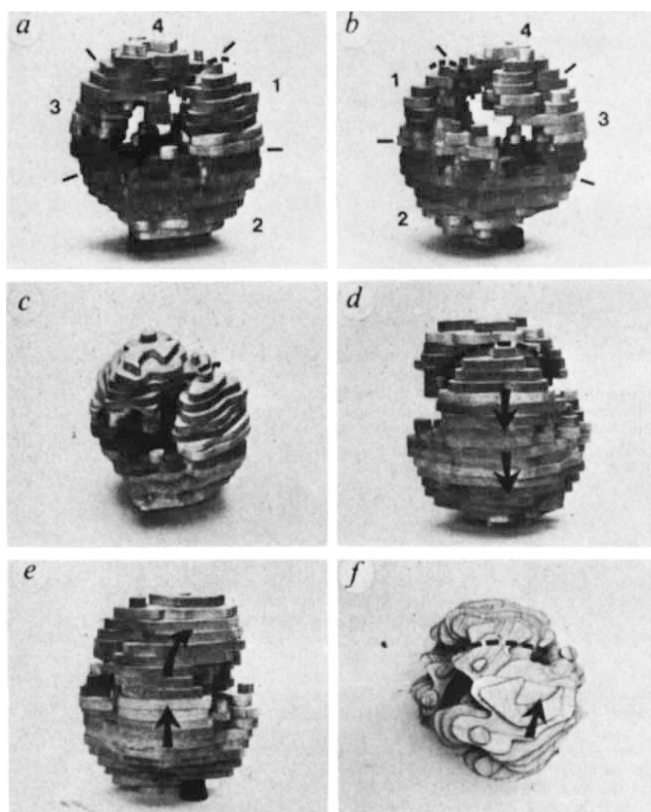


Fig. 4 Average structure of the reconstructed BR RNP particle. *a*, Front view of the particle corresponding to Fig. 4e. The right- and left-hand sides of the particle are defined from the view seen in *a*. In *b*, the particle is viewed on from the back, in *c* from the front and above, in *d* from the right, in *e* from the left and in *f* from the top. The slit in the ring-like structure is indicated by a broken line. In *a* and *b* the four domains have been demarcated with numbers and division lines. Arrows in *d-f* denote the skewed course of the ribbon-like RNP element beginning in domain 1 and ending in domain 4.

a rather sharp bend and extends almost half-way up on the other side, still keeping about the same width (Fig. 4e). Taken as a whole, domain 2 can be described as an asymmetrical, bowl-like structure (Fig. 4c). The transition to domain 3 at 8 o'clock is again distinct; the RNP element gets narrower and attains a width of 250 Å until finally, at 11 o'clock, the RNP element bends to the right (Fig. 4e) and passes into domain 4, which has a maximal width of 350 Å and forms a more irregular structure (Fig. 4f). The major part of domain 4 is dislocated to the right in Fig. 4f, which explains the earlier notion of the particle as a short, slightly left-handed spiral²³. Domain 4 extends towards the slit and completes the ring-like structure.

Visualization of RNP particle formation on the gene also allows us to decide the order in which the four domains are synthesized. Three consecutive growth stages of the particle, as

well as a completed particle, are displayed in Fig. 5. The newly synthesized RNA fibre passes a transient thick fibre state, the stalk, before it is built into the dense portion of the RNP particle²³. The dense RNP element on top of the stalk is gradually extended (Fig. 5a-c) and attains the structure of an open ring with the same dimensions as the completed particle (Fig. 5d), which suggests that the RNP element of the particle is gradually completed from one end to the other. Towards the 3' end of the gene about three-quarters of a completed RNP particle can be seen on top of the stalk (Fig. 5c). Detailed comparisons with the three-dimensional reconstructed particle (Fig. 4b; see also Fig. 5d) show that the structure of the growing dense RNP particle is compatible with the assumption that the smooth end of domain 1 represents the leading edge, and that the thick domain 2 as well as the relatively thin domain 3 gradually become incorporated into the growing, dense RNP structure (Fig. 5a-c). The main alternative, that domain 4 is in the lead followed by domains 3 and 2, could not satisfy the projections of the growing particles observed. It is likely that domain 4 is formed from the stalk retracted towards the particle on release of the particle from the template. The established order of synthesis of the domains suggests that the 5' end of the RNP transcript is located at the top of domain 1 whereas the 3' end should reside somewhere in domain 4.

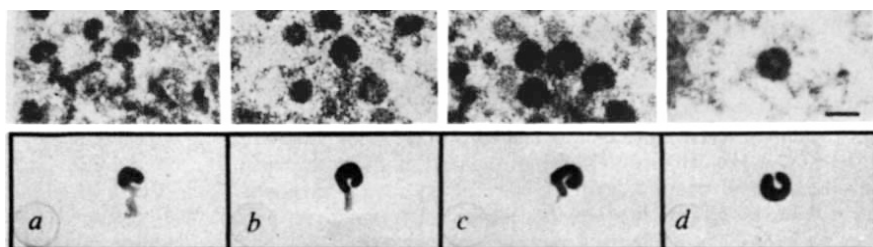
Functional implications

We have shown here that the BR RNP particle does not attain a random higher-order structure, but rather a well-defined, asymmetrical configuration comprising four major domains. This distinctive structure is probably closely related to the fate of the BR RNP particle, in particular to the transport and processing of the pre-messenger RNA molecule.

The structure of the particle could be relevant to the transport process for several reasons. The particle is remarkably compact with the dense RNP ribbon moulded into a spherical shape. Defined regions, or domains, of the particle may have specific recognition and binding functions during transport, for example, when the particle is associated with the fibrous network in the nuclear sap (see Fig. 1), or when it recognizes the nuclear pore. Finally, when the particle passes through the pore, it changes its ring-like configuration to an extended rod-like structure^{24,25}. This translocation event could be dependent on the specific organization and domain structure of the particle.

One or more of the domains could be designed to participate directly in the processing of the pre-messenger molecule. A considerable portion of the BR RNA molecules in the nuclear sap become polyadenylated³³ and are also likely to be spliced. It is known that a BR 1 gene harbours a 55-base-pair (bp)-long intron ~600 bp from the poly(A) site³⁴. As the members of the BR gene family display a strikingly similar sequence organization^{35,36}, it seems reasonable to assume that the other BR 75S RNA genes also carry an intron in the same position. In addition, U1 and U2 snRNP particles appear in all the BRs³⁷. As there is good evidence that both U1 snRNP³⁸⁻⁴⁰ and U2 snRNP⁴¹ are involved in the splicing process, this observation also supports the view that the BR transcripts are spliced. The 55-bp intron is close to the 3' end, which suggests that it resides in the particle in domain 4 (which contains about a quarter of the 37-kilobase

Fig. 5 Electron micrographs and schematic drawings of three growing BR particles (*a-c*) and of one completed and released particle (*d*). Bar, 500 Å.



(kb)-long transcript²¹). We conclude that polyadenylation and splicing occur in domain 4 of the BR particle.

Because of the ring-like configuration of the particle, domain 4 containing the 3' poly(A) tail and the intron ends up close to domain 1 with the 5' end of the transcript. This spatial relation could be fortuitous, but the cap structure is important for the polyadenylation and splicing processes *in vitro*^{42,43}. The cap structure may exert its effect as an organizer of the RNP particle as an entity, but it could also interact more specifically with adjacent structures of the RNP particle. Further analyses of

both spliceosomes in the *in vitro* systems^{42,44-46} and specific pre-messenger RNP particles *in situ* are likely to shed more light on the nature and interdependence of the steps in the processing of pre-messenger RNA into mature mRNA.

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LETTERS TO NATURE

A quasar with $z = 3.71$ and limits on the number of more distant objects

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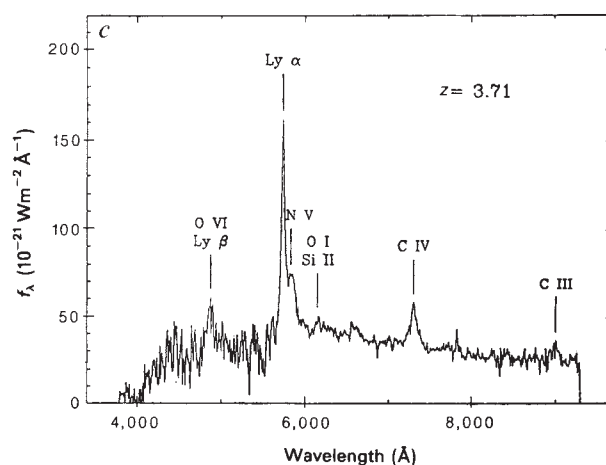
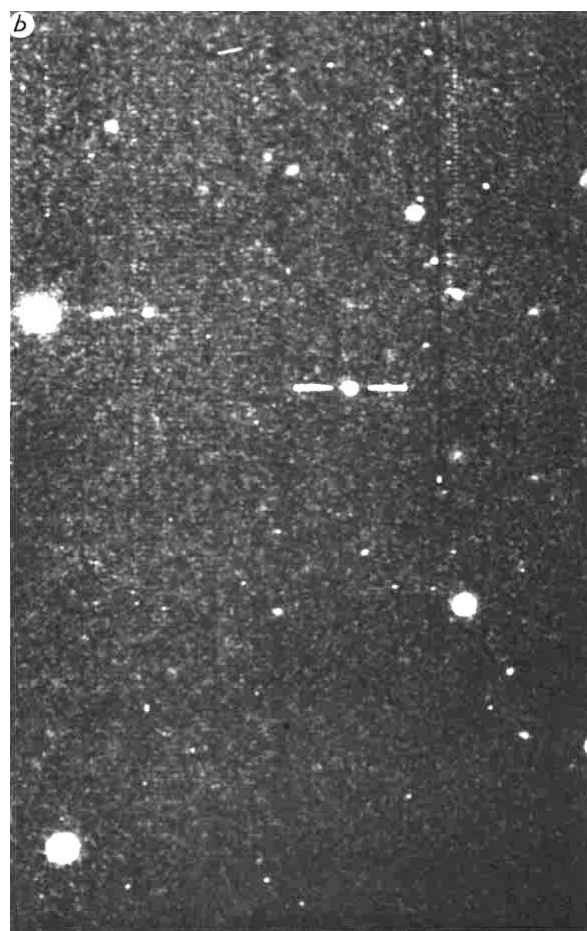
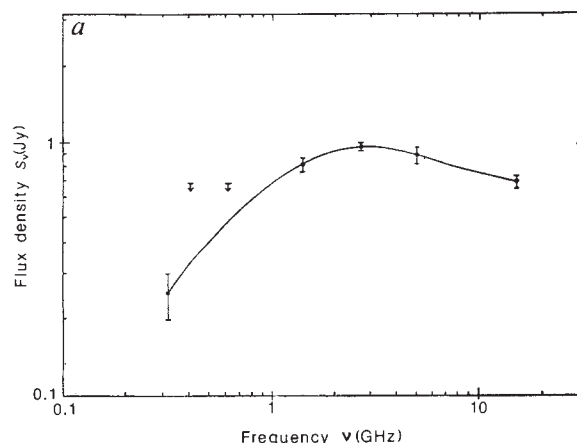
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Spectroscopy of objects identified with radio sources from the Parkes Selected Regions has shown the compact, flat-spectrum source 1351-018 to be a quasar with redshift 3.71, the second most distant discovered to date. Compared with known quasars at such high redshifts, the object is optically very faint ($B = 20.9$), red in colour ($B - R = 1.6$) and is distinguishable photometrically from faint galaxies only by its relatively blue optical-infrared colour ($R - K = 2.1$). In common with other high-redshift, radio-loud quasars, 1351-018 has weak emission lines when compared with objects selected optically through objective-prism or grism techniques. Moreover, weak-lined objects such as this may also be missed even by surveys using broad-band optical colours, which may help to explain why such searches have failed to find faint, high-redshift quasars. Because it is a member of a complete sample, 1351-018, in fact, confirms the recent suggestion¹ that the quasar radio luminosity function declines for redshifts $z > 2$. Extrapolation of the existing data predicts that the main Parkes Survey may contain about eight flat-spectrum quasars with $z > 4$.

The radio source 1351-018 lies in one of the deepest areas of the Parkes survey, the six $6.5 \times 6.5^\circ$ Selected Regions, which are complete to flux densities $S > 0.1$ Jy at 2.7 GHz (ref. 2). We are carrying out a detailed optical study of the 178 radio sources in the complete sample defined by these regions, with the aim of determining the high-redshift evolution of the radio source population. The 'intermediate' flux density means that powerful radio galaxies and quasars such as those found in bright, complete samples at $z \sim 1$ will be included in this sample up to $z \sim 4$, while the high selection frequency (2.7 GHz) makes possible the study of both steep- and flat-spectrum sources, the latter appearing in very small numbers in low-frequency surveys such as 3CR. We have completed the first phase of the programme—optical identifications on UK Schmidt plates, using extensive Very Large Array (VLA) observations to define accurate expected positions for optical counterparts. At this level, 56% of the sources are identified³. We now report the results of both deep charge-coupled device (CCD) imaging of the remaining empty fields and spectroscopic observations of sky-survey identifications. The first night of the spectroscopic programme yielded, among others, the spectrum of the quasar 1351-018, thus confirming the ability of the sample to probe high-redshift space.

PKS1351-018 is a VLA calibrator which is unresolved at 0.1 arc s resolution. The radio spectrum (Fig. 1a) has a broad peak over two decades in frequency, similar to that found in some other high-luminosity compact quasars⁴. Previous attempts at optical identification were initially unsuccessful⁵ but later resulted in the classification of 'quasar'⁶. We now confirm the latter identification by position coincidence: a faint red object is visible near the limits of the Palomar Sky Survey E plate and on the UK Schmidt J plate at RA 13 h 51 min 32.00 s, dec. $-01^\circ 51' 20.0 \pm 0.3''$ (1950.0), but it is too near the plate limits for its nature to be determined morphologically. The red colour, typical of faint radio galaxy identifications, was subsequently

Fig. 1 *a*, The radio spectrum of PKS1351-018 (ref. 3). *b*, The optical counterpart of PKS1351-018, indicated by two bars in the centre of the field. The figure is reproduced from a *B*-band CCD image, and shows an area 2.5×4 arc min, with north to the top and east to the left. *c*, The optical spectrum of PKS1351-018 obtained on 20 April 1985 with the FORS and the RGO Spectrograph at the *f*/8 focus of the Anglo-Australian Telescope. The combined spectrum has a resolution of ~ 20 Å; the blue half was smoothed to a resolution similar to that of the red side, and the two spectra were joined at 5,300 Å. The absolute flux calibration was determined from the CCD *B* magnitude.



confirmed by our CCD photometry performed at the Anglo-Australian Telescope in April 1985 which gave a *B* magnitude of $20.89 (\pm 0.03)$ with $B - R = 1.6 (\pm 0.1)$. The Kitt Peak National Observatory (KPNO) interference filter system was used with red standards to zero-point the colours to the Cousins system. Figure 1*b* shows the *B* band CCD image taken in a 200-s exposure with the Royal Greenwich Observatory (RGO) CCD camera.

Ambiguity concerning the nature of the optical object was removed by observations with the United Kingdom Infrared Telescope (UKIRT) on 20 March 1985. An infrared study of the Parkes Selected Regions is being undertaken to enable an extension of previous work on the infrared properties of radio galaxies^{7,8}, both to fainter flux densities and to compact radio sources. These observations gave infrared magnitudes of $K = 17.26 \pm 0.19$ and $J = 18.32 \pm 0.18$ (on the Cerro Tololo Inter-American Observatory system) for 1351-018 and established it as a quasar candidate by virtue of its optical-infrared colour. This object lies among established quasars on the $(R - K)/K$ colour-magnitude diagram (Fig. 2), considerably to the blue of the well-defined radio galaxy locus. The combined properties of compact, flat-spectrum radio source, faint, red optical object, and blue optical-infrared colour thus made 1351-018 a prime candidate for a high-redshift quasar.

The optical spectrum (Fig. 1*c*) was obtained on the night of 20 April 1985 and is the result of a 1-h integration using the Faint Object Red Spectrograph (FORS) in tandem with the Image Photon Counting System (IPCS)/RGO Spectrograph at the *f*/8 Cassegrain focus of the Anglo-Australian Telescope. The observations used a 1.3-arc s slit with dichroic splitting of the beam at 5,300 Å. The grism produced a FORS spectrum with a resolution of 20 Å with the resolution in the blue camera roughly a factor of three higher. The wavelengths of the main emission features and their identifications are given in Table 1. In common with other high-redshift quasars, Ly α is the strongest line with C III, C IV, N V and O VI also clearly present. The mean redshift based on the emission lines of C III, C IV, N V, O VI, and O I/Si II is 3.709 ± 0.003 . Ly α was not used as the strong forest of absorption lines blueward of Ly α biases the centroid of the Ly α emission line to high redshifts.

The fact that 1351-018 is 2.3 mag fainter than PKS2000-330 ($z = 3.78$) casts some doubt on recent suggestions that searches deeper than a magnitude of $R = 18$ would be unlikely to result in the discovery of high-redshift objects⁹. With an optical absolute magnitude of $M_B = -26.8$ (assuming a Hubble constant of $50 \text{ km s}^{-1} \text{ Mpc}^{-1}$, a density parameter $\Omega_0 = 1$ and flux density varying as $\nu^{-0.5}$ beyond 9,000 Å), 1351-018 is the only known quasar at $z > 3.5$ to have an optical luminosity comparable with that of typical low-redshift radio-loud quasars. However, extrapolation of the radio-quasar equivalent of the Hubble diagram¹⁰ suggests that the magnitude of 1351-018 is typical of that expected for a quasar at $z \approx 4$; high-redshift quasars in radio samples are expected to lie mainly among the fainter optical identifications.

Given the unusual nature of 1351-018, it is interesting to ask whether similar objects could be found using purely optical selection criteria. Comparison of the spectra of high-redshift quasars reveals 1351-018 (and other radio-selected quasars such as PKS2000-330)⁴ to have systematically weaker emission

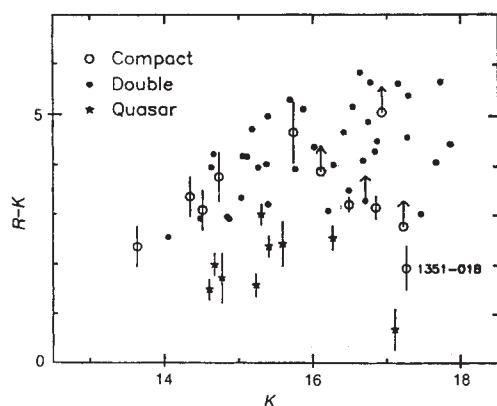


Fig. 2 The $(R-K)/K$ optical-infrared colour-magnitude diagram showing the positions of optical objects associated with different classes of radio source. Our data on Selected Region sources have been augmented by data on classical-double radio galaxies^{7,8}, and with additional quasar data^{15,16}. The compact radio source 1351-018 lies in the region occupied by optical objects which are established quasars. Error bars are derived from the original plate photometry to indicate that such accuracy was adequate for selection of 1351-018.

lines than their optically selected counterparts^{11,12}. An estimate of the rest-frame-equivalent width (W_0) of Ly α in 1351-018 yields $W_0 = 35 \pm 8 \text{ \AA}$, compared with $W_0 = 88 \text{ \AA}$ in 0055-2659 (the most distant optically selected quasar, $z = 3.68$)¹¹. Such a bias is not unexpected in prism or grism plate surveys, but, in fact, may also affect colour-based studies such as those of Koo¹³. Using the flux-calibrated spectrum of 1351-018, we can synthesize the colours of this object for various filter systems and also work out the variation of these colours with redshift. The synthesized $B-R$ colour agrees with the photometrically measured value to within 0.1 mag, suggesting that using the spectrum in this manner is satisfactory. Using Koo's UJF filter system we find synthesized $U-J$ and $J-F$ colours of 2.7 ± 0.5 and 1.4 ± 0.1 respectively, the relatively large uncertainty in $U-J$ being due to the very small flux in the U band. We have also investigated the locus followed by 1351-018 on the $U-J/J-F$ colour-magnitude diagram as it varies in redshift, allowing for changes with redshift in blanketing of the Lyman continuum. The $(U-J, J-F)$ colours are $(0.4, 0.5)$ at $z = 3$ and $(1.8, 1.2)$ at $z = 3.5$, which would not be classified as significantly non-stellar in Koo's faintest magnitude bin. Between $z = 3$ and $z = 3.5$, the track of 1351-018 departs from the stellar locus, but for $z > 3.5$ and $2.7 < z < 3$, faint quasars similar to 1351-018 would not have been detected. Furthermore, the spectrum of 1351-018 falls rapidly just blueward of the Lyman limit, whereas many high-redshift quasars retain significant flux at this point (for example, 0055-2659). Adding a significant fraction of ultraviolet flux to 1351-018 would make its $(U-J, J-F)$ colours stellar at all $z > 2.5$. Conversely, by increasing the strength of the main emission lines in 1351-018, we can simulate the colours of a stronger-lined object, which we find to be clearly non-stellar at any redshift for an increase by a factor ~ 2 in line strength.

It therefore seems feasible that *both* prism-plate and colour selection of high-redshift quasars may be biased towards bright, strong-lined objects. If we hypothesize that most high-redshift quasars might have spectra similar to 1351-018, then the failure of faint surveys to find significant numbers of high-redshift objects could be largely a result of the selection criteria used. The conclusion that quasars were fewer but brighter in the past^{11,13} may be correct but is based on searches for optically faint quasars at high redshifts which are incomplete for objects such as 1351-018. Note that all high-redshift quasars should be distinguishable on the basis of non-stellar colours, provided

Table 1 Emission lines in PKS1351-018

Observed wavelength ($\text{\AA}$)	Identification ($\text{\AA}$)	Redshift
4,870	[Ly β 1,025.7	3.748]
	O VI 1,033.8	3.711
5,735	Ly α 1,215.7	3.717
5,830	N V 1,240.1	3.701
6,155	[O II 1,303.5	3.722]
	Si II 1,307.6	3.707
7,295	C IV 1,549.1	3.709
9,005	C III 1,909.0	3.717

Table 2 Values of $\langle (V_e - V_0)/(V_a - V_0) \rangle$ for various values of z_0

z_0	n	$\langle (V_e - V_0)/(V_a - V_0) \rangle$ ($\Omega_0 = 1$)	$\langle (V_e - V_0)/(V_a - V_0) \rangle$ ($\Omega_0 = 0$)
1.8	30	0.375	0.424
1.9	28	0.321	0.359
2.0	22	0.304	0.345
2.1	17	0.301	0.345

near-infrared (J, H, K) data are utilized in the selection procedure.

1351-018 lies in one of the two Parkes Selected Regions (centred on RA 12 h 04 min 50 s, dec. $-00^\circ 31'$ and RA 13 h 40 min 48 s, dec. $-00^\circ 29'$) on which extensive CCD imaging has been performed. This subset (55 sources) of the main sample (178 sources) is now provisionally 96% identified and can be used to draw some preliminary conclusions. Extrapolation of the observed evolution in the radio luminosity function at $z = 1$ to higher redshifts predicts that $> 20\%$ of steep-spectrum sources at our flux-density level should lie at $z > 2$ (ref. 1), unless the co-moving density of objects falls at higher redshifts. With only one steep-spectrum source in the Selected Regions subsample remaining unidentified at $R \sim 24$, we now have the first indication of a redshift cutoff for these radio galaxies, since galaxies above this limiting magnitude should have redshifts $z < 2$. Extension of CCD imaging to the other regions will be needed to increase the confidence in this result.

Here we wish to concentrate on the subset of flat-spectrum sources in the Selected Regions which can be used as the faint end of a combined sample utilizing all the available complete-sample data on this class of source. Combining existing brighter datasets¹ with the 12-13 h regions discussed here, we can construct a total sample at 2.7 GHz of 151 flat-spectrum sources, of which 147 are optically identified and 119 have known redshifts. The unknown redshifts may be roughly estimated from apparent magnitude¹.

A useful way of quantifying evolution is the V_e/V_a statistic (where V_e and V_a are the cosmological volumes enclosed by, and available to, a source in the combined sample)¹⁴, which allows analysis of such a combined sample in a generalization of the V/V_{max} test. To study evolution in specific redshift ranges, a banded version of this is required to prevent evolution at other redshifts influencing the result. We therefore evaluate $\langle (V_e - V_0)/(V_a - V_0) \rangle$ (where V_0 = cosmological volume enclosed by a redshift z_0), to study evolution beyond a redshift of z_0 . The results for various values of z_0 are given in Table 2. The low values of $\langle V_e/V_a \rangle$ (that is, < 0.5) reinforce the recent suggestion¹ of a deficit of high-redshift flat-spectrum objects. The basic result can be incorrect only if most objects with unknown redshift lie at $z > 3$. This seems implausible because they are generally neither very red nor faint.

To illustrate the fall in co-moving density at high redshift, we have fitted a simple parametric model of the flat-spectrum radio luminosity function to the available data. Limiting consideration to $z > 1.8$ provides a data set of 30 objects, to which we can fit a model of the form $\rho(P, z) \propto P^{-\beta} (1+z)^{-\gamma}$ (where $\rho =$

co-moving density per unit interval of P , and P = luminosity in $\text{W Hz}^{-1} \text{sr}^{-1}$; the maximum-likelihood values for the parameters are $\beta = 2.8$ and $\gamma = 3.1$ ($\Omega_0 = 1$ only was considered) corresponding to a reduction in ρ of a factor ~ 5 between $z = 2$ and $z = 4$. Extrapolating this model, we estimate that the main Parkes survey may contain ~ 8 flat-spectrum quasars with $z > 4$. To put this in context, there are $\sim 1,200$ flat-spectrum sources in the survey, of which more than half lack redshifts. If the true number were, in fact, zero, we would conclude that the luminosity function falls still more quickly at high redshifts. Nevertheless, it seems reasonable to suggest that $z > 4$ quasars might be found, and that these objects, like 1351-018, will lie among the faint, red optical identifications of compact radio sources.

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Will cosmic strings be discovered using the Space Telescope?

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Cosmic strings are topologically stable defects in the vacuum which may be produced at a phase transition in the early Universe. Some may form closed loops, and some may be open, extending over the entire observable Universe. Although gravitational lensing of distant quasars by cosmic strings has been discussed¹⁻⁶ it is difficult to prove that a particular pair of quasar images is formed by a cosmic string. We suggest here that observations of very distant galaxies are more useful as a means for their discovery. A galaxy may appear as cut by a sharp edge if there is a cosmic string between the galaxy and the observer. We argue that if there is only one string out to a redshift $z \approx 1$, then the probability that it crosses a random image obtained with the Wide Field Camera (WFC) of the Space Telescope is $\sim 10^{-4}$. Therefore, there is a fair chance of discovering a string, after taking 10^4 images. The WFC will operate almost continuously in primary and serendipity modes, and a cosmic string, if it exists, may be discovered using the Space Telescope within the first few years of its operation.

If there is just one open cosmic string within the horizon, then the probability that the nearest part of it is at a redshift $z < 1$ is about 0.1 (ref. 6). The total length of all cosmic strings, including the loops, is logarithmically larger^{4,6}, so the probability of having some strings closer than at a redshift $z = 1$ is close to one. A string may lens a few quasars and separate the two images by up to a few arc minutes^{4,6}.

A number of known pairs or triplets of quasars have almost identical redshifts and angular separations (θ) between 1 and

4 arc min: Hazard 1146+111 B, C ($z = 1.01$, $\Delta z = 0.001$, $\Delta\theta = 2.6$ arc min)⁷, Q0952+698 A, B, C ($z = 2.05$, $\Delta z = 0.014$, $\Delta\theta = 3.6$ arc min)⁸, Q0307-195 A, B ($z = 2.13$, $\Delta z = 0.022$, $\Delta\theta = 1.0$ arc min)^{9,10}, U2 and U3 near NGC2683 ($z = 1.26$, $\Delta z = 0.010$, $\Delta\theta = 3.6$ arc min)¹¹, Q0107-025 A, B ($z = 0.96$, $\Delta z = 0.004$, $\Delta\theta = 1.3$ arc min)¹². These might be single quasars lensed by clusters or superclusters of galaxies^{13,14}, or in case of the pairs, these may be single quasars lensed by cosmic strings, or these may be separate quasars located in clusters or superclusters of galaxies^{7,15}. How do we choose between these possibilities?

The main reason for favouring the picture with a few separate quasars located in the distant clusters or superclusters, over the gravitational imaging, is the presence of a small but significant discrepancy in redshifts between the components of all those pairs or triplets, typically $\sim 1,000 \text{ km s}^{-1}$. However, if the separation between multiple images is a few arc minutes, then the time delay between various light paths is in the range of 100-1,000 yr. We have no direct observational or theoretical evidence on how stable are redshifts of quasars on such a time scale. There is some indirect observational evidence that considerable variations in the measured radial velocities might be expected. Broad emission lines in Type I Seyferts and in quasars are frequently asymmetrical, and the broad line peaks are shifted with respect to narrow lines by as much as $\pm 2,000 \text{ km s}^{-1}$ (refs 16-19). These shifts may vary, perhaps in 100 or 1,000 yr, and these time variations may be responsible for the redshift differences between the two images.

The following approach may resolve the present ambiguity about the nature of 'clusters of quasars'. Quasars also have forbidden emission lines which are much narrower than the broad lines²⁰. They also show asymmetries²¹, but systematic errors in measured redshifts may be smaller due to a smaller linewidth. Also, it is believed that forbidden lines form in regions which are a few hundred parsecs across, so their profiles should be more stable. The strongest line is [O III]5,007, a much weaker one is [O II]3,727. Both lines are redshifted into near infrared, and may be difficult to observe. However, if accurate redshifts and profiles were obtained for those lines then the present ambiguity might be resolved. If the redshifts obtained with forbidden lines are the same for both images then the case for gravitational lensing is stronger. If the redshifts are different then a cluster of quasars is more likely.

Suppose, the proposed program is carried out, and we become convinced that some widely separated pairs of quasars are, in fact, gravitationally lensed. We still would not know if the lensing were due to a cosmic string, to a cluster of faint galaxies, or to some other object. It is hard to think of a test that would demonstrate that a given pair of point-like quasars is, in fact, a single object lensed by a cosmic string, and not by something else.

The situation is entirely different for an extended object, such as a galaxy. Lensing by another galaxy, a cluster of galaxies, or a single massive object could produce multiple images and distort every image. However, some cosmic strings are expected to be straight^{1,4,6}, and their gravitational lensing would have a very striking signature: a background galaxy would look as though it had a sharp edge. No galaxies are known to be distorted in such a way, and the sharp edge would be a very clear indication that the galaxy is lensed by a cosmic string. By extrapolating the edge in two directions one should be able to see the same string cutting across images of other distant galaxies.

It is not clear how large the image of a galaxy should be in order to detect a sharp edge. Probably, the galaxy must be large enough to make a morphological classification possible: there should be at least 10 pixels across its image.

No cases of galaxies with sharp edges are known in the Palomar Sky Survey. As the survey covers two-thirds of the sky, this implies that no cosmic strings capable of large gravitational image splittings were discovered out to the redshift $z \approx 0.02$, or

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so. High-resolution charge-coupled device (CCD) images obtained using the biggest telescopes cover an extremely small fraction of the sky, and do not put useful constraints on the presence of cosmic strings: even at the best CCD frames the galaxies cannot be classified by their shape beyond $z \approx 0.1$.

The Space Telescope will make a dramatic improvement. Morphological classification of galaxies will be possible out to a redshift $z \approx 1$ (ref. 22), and, therefore, sharp edges in galaxy images should be detectable out to that redshift. The ground-based observations show that there are 16,000 galaxies brighter than 24 mag in the *J* band per one square degree²³, and the number increases by a factor of 2.5 per magnitude. The WFC has a field-of-view 2.67×2.67 arc min (ref. 24). Therefore, there should be about 30 galaxies brighter than 24 mag, and about 200 galaxies brighter than 26 mag for each picture taken by the WFC on the Space Telescope. Any straight line which crosses the field has to cross many galaxies. It is hard to tell what fraction of those galaxies will be at a redshift > 1 , but it is likely to be appreciable. I shall adopt an optimistic, but not implausible, view that if a string crosses the picture then there will be at least one very large redshift galaxy crossed by the string, and it will display a sharp edge. Thus I assume that if a string is in the field-of-view, its presence may be detected with a probability close to unity.

Let us assume that there is just one cosmic string with 100° segment close enough to have background galaxies detectable with the Space Telescope. The WFC must have the centre of its field-of-view within about 1.5 arc min of the string in order to 'see' the string. This gives the effective area for the string detection of $S \approx 100^\circ \times 3' = 5^\circ$, roughly 10^{-4} of the whole sky. Therefore, an image taken randomly with the WFC will have a probability of 10^{-4} of falling into this strip on the sky, that is, there is a 10^{-4} probability that the string will be within the field-of-view of a random picture. About 10^4 deep images are required to have a reasonably large probability to detect the presence of the string. That many images will be obtained within the first few years of operation of the Space Telescope.

If the average image taken with the WFC shows many large redshift galaxies, then the method proposed here will be much more straightforward than the search for lines of double galaxies⁴. The angular separation between the two images of a galaxy lensed by a given cosmic string depends on the redshift of the galaxy⁶. Therefore, the emerging pattern is likely to be complicated and difficult to decipher.

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Determination of cosmogenic ^{41}Ca in a meteorite with tandem accelerator mass spectrometry

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Several long-lived cosmogenic radioisotopes— ^{14}C , ^{36}Cl , ^{26}Al , ^{10}Be , ^{53}Mn and ^{129}I —have been used to investigate the history of meteoric material and lunar samples. One of the most interesting results has been the determination of the residence time or 'terrestrial age' of meteorites found on ablation areas of the Antarctic ice sheets. As these ages range up to 700,000 years¹, ^{36}Cl , with a half-life of 3.01×10^5 yr has been the most useful isotope, but for reliable measurements of terrestrial ages below 300,000 yr, a radioisotope with a half-life between those of ^{36}Cl and ^{14}C ($T_{1/2} = 5730$ yr) is needed. Calcium-41 is a suitable candidate, with $T_{1/2} = 1.03 \times 10^5$ yr, but it occurs in concentrations below the detection limit of conventional techniques. Here we report the first use of tandem accelerator mass spectrometry (TAMS) to measure ^{41}Ca in a natural sample—the Bogou iron meteorite. We find a $^{41}\text{Ca}/\text{Ca}$ ratio of $(3.8 \pm 0.6) \times 10^{-12}$ which corresponds to a ^{41}Ca activity of 6.9 ± 1.1 d.p.m. per kg meteorite. This implies that ^{41}Ca can be successfully used to determine the irradiation history of meteorites.

Calcium-41 has been proposed as a dating tool in palaeo-chronology and in dating CaCO_3 sediments². ^{41}Ca is produced on the surface of the Earth by neutron activation of ^{40}Ca ; this dominates atmospheric production rates because of the low abundance of sufficiently heavy targets in the atmosphere. The calculated saturation concentrations $^{41}\text{Ca}/\text{Ca}$ in the terrestrial environment are between 10^{-15} and 10^{-14} (ref. 2). Accelerator mass spectrometry is the only way to measure these low concentrations. Decay counting is not very sensitive because of the long lifetime of ^{41}Ca and because ^{41}Ca decays only by electron capture (the 3.3 keV X rays of ^{41}K are difficult to detect). The current detection sensitivity for accelerator mass spectrometry, $^{41}\text{Ca}/\text{Ca} \approx 10^{-13}$, is still not sufficient by two orders of magnitude to measure terrestrial samples, but is high enough to measure meteorites. ^{41}Ca in meteorites is produced by cosmic ray spallation, mainly of Fe and Ni, and by the capture of thermal neutrons by ^{40}Ca .

Many of the meteorites studied with TAMS have been selected from the thousands of meteorites collected from ablation areas of the Antarctic ice sheet. Stable noble gas isotopes are used to identify meteorites with exposure times long enough to ensure saturation of the radioisotope of interest before the meteorite fell onto the earth. If the longer-lived radioisotopes ^{53}Mn ($T_{1/2} = 3.7 \times 10^6$ yr) and ^{10}Be ($T_{1/2} = 1.6 \times 10^6$ yr) are not found to be saturated, the meteorite may have originated inside a larger body that broke up following a collision in space or on entry into the earth's atmosphere^{3,4}. If ^{53}Mn and ^{10}Be are saturated, a measurement of ^{14}C , ^{41}Ca and/or ^{36}Cl determines the terrestrial age of the meteorite.

The ^{41}Ca measurements were carried out with the Rochester tandem Van de Graaff accelerator. The apparatus and the general method of measuring radioisotope ratios at Rochester are described elsewhere⁵ together with some initial ^{41}Ca results. Details of the method of measuring ^{41}Ca are reported here.

Negative CaO^- ions were produced in our scanning Hiconex (made by the General Ionex Corporation, Newburyport, Massachusetts, USA) caesium sputter ion source. Acceleration of CaO^- ions was chosen over CaH_3^- ions even though the latter results in a lower ^{41}K interference^{6,7}. This was done because it

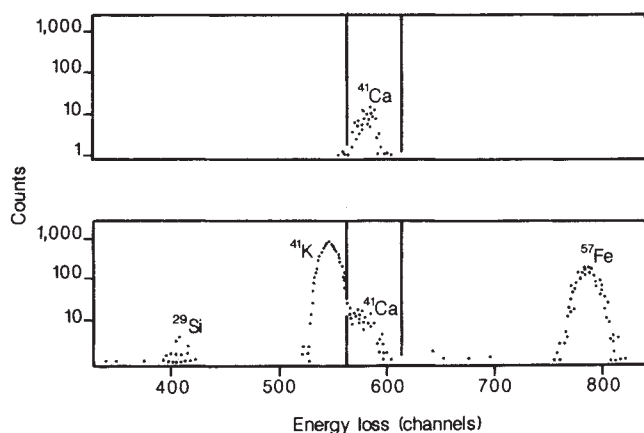


Fig. 1 ΔE spectra taken from the second plate of the ionization detector. The upper spectrum shows events that passed software gates set on four ΔE signals and the total energy signal. The lower spectrum shows the ungated events. The use of a new high-resolution injector⁵ eliminated interference from $^{42}\text{Ca}^{16}\text{O}^-$. When the injector magnet was set for mass 57, a small fraction of the high-energy tail of $^{40}\text{Ca}^{16}\text{O}^-$, all of $^{40}\text{Ca}^{17}\text{O}^-$ ($\approx 4 \times 10^{-4}$ of ^{40}CaO), and all of $^{41}\text{K}^{16}\text{O}^-$ was transmitted into the accelerator. Except for ^{41}K , the mass resolution of the system was good enough to remove these interferences, but there was a low counting rate from two other ions having nearly the same M/q . The small ^{29}Si peak (charge state 6+) originated from $^{28}\text{Si}^{29}\text{Si}^-$ molecules (the sample holder was made out of Si). The ^{57}Fe peak (charge state 11+) comes from stainless steel parts in the ion source. These two ions have nearly the same magnetic and electric rigidity as the ^{41}Ca in the charge states indicated but require at least one scattering or charge-changing event to be able to reach the detector, where they are well separated.

is very difficult and somewhat inefficient to prepare the small and pure metallic Ca samples necessary for producing adequate beams of CaH_3^- ions. Our CaO samples had high enough radioisotope ratios and contained low enough K to allow separation of ^{41}K from ^{41}Ca in our gas ionization detector⁵. The terminal voltage was set at 10 MV. Charge state $q=8$ was chosen, since this gave the best yield from the terminal stripper foil. The $^{41}\text{Ca}/\text{Ca}$ ratios were determined by alternately measuring the ^{40}Ca beam current in the image Faraday cup following the accelerator and the ^{41}Ca particles in the gas counter following a 10° electrostatic analyser. Figure 1 shows a ΔE spectrum taken with the gas detector. The data analysis procedure is described in ref. 8.

The Bogou meteorite fell on 14 August 1962 in Bourkina-Fasso (Africa). The recovered mass was 8.8 kg. The following chemistry was performed to extract the calcium and to remove potassium: after adding 6.0 mg of Ca carrier, 636 mg of the Bogou meteorite was dissolved in dilute HNO_3 . About 2% of the solution was taken as an aliquot for chemical analysis by atomic absorption spectroscopy. After separate hydroxide precipitation, 4% ammonium oxalate was added to the supernatant. The calcium oxalate was separated and dissolved in diluted HCl. The Ca was then purified through a cation exchange resin (AG 50W-X8) and precipitated as calcium oxalate. The precipitate was ignited to CaO in a quartz vial at about $1,100^\circ\text{C}$ for 10 min. The chemical procedures and analysis methods for ^{10}Be , ^{36}Cl and ^{53}Mn , which were also measured in the Bogou meteorite, were essentially the same as those reported earlier^{1,3,9}.

The results (Table 1) show that $^{41}\text{Ca}/\text{Ca}$ ratios in the 10^{-12} range can be measured with uncertainties of about 15%. The present background ratio for a chemistry blank is an order of magnitude lower. Improvements in the chemical removal of potassium from the samples and from the caesium should be possible. This will lower background ratios and may allow us to measure ratios in the 10^{-13} range. However, the present

Table 1 Results of ^{41}Ca measurements

Sample	^{41}Ca counts	$^{41}\text{Ca}/\text{Ca}$ (10^{-12})	^{41}Ca atoms per g (10^8)	^{41}Ca d.p.m. per kg
Bogou	93	3.8 ± 0.6	5.4 ± 1.0	6.9 ± 1.1
Blank	6	0.18 ± 0.08		
Standard		95.6 ± 6.1		

The ^{41}Ca standard was prepared by diluting the ^{41}Ca counting source which was used for the ^{41}Ca half-life measurement of Mabuchi *et al.*¹¹. The given uncertainty is that of their ^{41}Ca half-life ($T_{1/2} = 1.03 \times 10^5$ yr). All samples (meteorite, standard, chemistry blank) were mixed with Ag powder ($\text{CaO} : \text{Ag} \approx 1 : 4$ by weight) before loading on a wheel made of ultra-pure Si. The $^{41}\text{Ca}/\text{Ca}$ ratio of the sample was normalized to the ^{41}Ca standard after background subtraction. The total ^{41}Ca counting times for the meteorite and the blank sample were 46 and 85 min, respectively. The quoted uncertainties are one standard deviation.

Table 2 Cosmic ray-produced radionuclides in the Bogou meteorite

Nuclide	Half-life (yr)	Activity (d.p.m. per kg sample)
^{41}Ca	1.03×10^5	6.9 ± 1.1
^{36}Cl	3.01×10^5	22.2 ± 0.7
^{10}Be	1.6×10^6	5.1 ± 0.4
^{53}Mn	3.7×10^6	471 ± 20

limitation for producing lower ratios is not the interference from ^{41}K but the difficulty of producing higher negative ion currents and the small amount of sample available. About one third of the 5.9 mg meteorite CaO sample was consumed. This gives an overall detection efficiency for ^{41}Ca of about 10^{-7} . This could be improved with the use of a high intensity and high efficiency sputter source¹⁰. We hope that the use of such a source with our apparatus will make measurements of terrestrial samples possible.

We normalized the $^{41}\text{Ca}/\text{Ca}$ ratio to a standard produced from the sample of Mabuchi *et al.*¹¹. This is the sample that was used to obtain the generally accepted half-life of 1.03×10^5 years. Our earlier measurements⁵ indicated that this half-life may be too low. Any error in the Mabuchi half-life would most likely arise from their estimate of the $^{40}\text{Ca}(n, \gamma)^{41}\text{Ca}$ cross section or from their activity measurement. If the uncertainty is in the cross section, this would not affect our reported value of the ^{41}Ca activity, 6.9 ± 1.1 d.p.m. per kg. However, an error in their activity measurement would affect our reported activity by a proportional amount.

For a given radioisotope, the useful range for measuring terrestrial ages of meteorites can be determined from the experimental uncertainties. The terrestrial age and its uncertainty are calculated from

$$T = -\frac{1}{\lambda} \cdot \ln(R)$$

$$\Delta T = \frac{\Delta R}{R\lambda}$$

where $\lambda = \ln 2 / T_{1/2}$ and R is the ratio of measured activity to saturated activity. The uncertainty in the saturated activity is determined from the distribution in measurements for meteorites that fell recently and is usually 10–15%. At the low end of the age range, the uncertainty in R is usually dominated by the uncertainty in the saturated activity. For greater ages the activity is low and the uncertainty due to counting statistics becomes large. A young terrestrial meteorite age can be determined to $\pm 65,000$ yr using ^{36}Cl and, assuming comparable precision, we expect $\pm 23,000$ yr will be possible with ^{41}Ca . This results in approximate cross-over points of 40,000 yr for ^{14}C – ^{41}Ca and

about 300,000 yr for ^{41}Ca - ^{36}Cl (with considerable overlap at the boundaries).

Some conclusions can be drawn from a comparison of the cosmic ray produced radionuclides ^{10}Be , ^{36}Cl , ^{41}Ca and ^{53}Mn in the Bogou meteorite (see Table 2). The recovered size of the Bogou meteorite is about 7 cm in radius. The high ^{53}Mn concentration of 471 ± 20 d.p.m. per kg suggests a pre-atmospheric radius no larger than 15 cm. The ^{36}Cl value of 22.2 ± 0.7 d.p.m. per kg, which agrees well with the average saturation value of 22.8 ± 3.1 d.p.m. per kg (ref. 9), confirms this. Not enough ^{10}Be data have been tabulated for iron meteorites to use the measured value of 5.1 ± 0.4 d.p.m. per kg for an additional constraint on the radius of this meteorite.

Both ^{36}Cl and ^{41}Ca in an iron meteorite are high-energy spallation products and therefore their production should be depth-independent for a small-sized meteorite like the Bogou iron meteorite. There is little production from neutron capture because the ^{40}Ca concentration in an iron meteorite is less than 1 p.p.m. The measured spallation cross sections for ^{41}Ca (C. Perron, personal communication which revises data from ref. 12) are lower by about a factor of 1.5 ± 0.8 as compared with those of ^{36}Cl (ref. 13). Using the average ^{36}Cl saturation value, the estimated ^{41}Ca saturation activity should be 15 ± 8 d.p.m. per kg, which is larger than, but consistent with, the measured value of 6.9 ± 1.1 d.p.m. per kg. The closest analogue to ^{41}Ca in meteorites is ^{37}Ar , whose activity averaged over an 11-yr solar cycle is about 16 d.p.m. per kg Fe (ref. 14).

Although this is the first ^{41}Ca measurement in an iron meteorite, Mabuchi *et al.*^{15,16} have measured ^{41}Ca in stony meteorites using low level X-ray counting. Since ^{41}Ca in stony meteorites is produced from both Ca and Fe, these previous results can be used together with our result from an iron meteorite to obtain the production of ^{41}Ca from calcium. Using 100–200 g of meteorite Mabuchi *et al.* found 4.3 ± 1.9 d.p.m. ^{41}Ca per kg in the Granes meteorite and 48 ± 8 d.p.m. ^{41}Ca per kg in the Allende meteorite. The Granes meteorite contains 22.1% Fe, 1.35% Ca (ref. 15) and the Allende meteorite contains 23.85% Fe and 1.87% Ca (ref. 17). Using these values, we find that approximately 65% (95%) of the ^{41}Ca activity in Granes (Allende) comes from activation of ^{40}Ca . The fact that these numbers are very different in the two cases shows that ^{41}Ca is a useful nuclide for cosmic ray bombardment studies of meteorites. The ^{41}Ca activity in meteorites which are large enough to have a thermal neutron flux, such as Allende and Jilin¹⁸, should be easily measurable by accelerator mass spectrometry.

The successful measurement of ^{41}Ca in the Bogou meteorite with tandem accelerator mass spectrometry shows that this radioisotope can now be measured in extraterrestrial samples accurately enough to help determine irradiation histories of meteorites. Calcium-41 has a half-life that falls in the gap between ^{14}C and ^{36}Cl , a range that is needed for measuring terrestrial ages of Antarctic meteorites.

We wish to thank K. Notsu *et al.*¹¹ for providing the ^{41}Ca standard and the US National Museum for providing the Bogou meteorite sample. We thank T. Hemmick, R. Teng and the NSRL staff for technical assistance. This work was supported by NSF grant PHY-8214295 and NASA grant NAG 9-33.

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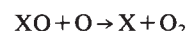
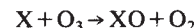
Detection of stratospheric N_2O_5 by infrared remote sounding

G. C. Toon, C. B. Farmer & R. H. Norton

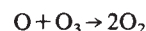
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The existence of stratospheric N_2O_5 , a temporary reservoir species whose photolysis products catalyse ozone destruction, has long been predicted¹ but has not previously been detected. We now report the identification of N_2O_5 absorption between 1,230 and 1,260 cm^{-1} in infrared spectra measured by the Atmospheric Trace Molecule Spectroscopy (ATMOS) experiment from orbit during the Spacelab 3 mission. Preliminary analysis of spectra recorded at sunrise on 1 May 1985 indicates a peak volume mixing ratio of 1.6×10^{-9} at 35 km altitude, or a broad concentration peak of 4×10^8 molecules cm^{-3} between 25 and 31 km. As expected, this absorption is not detectable in spectra measured at sunset because of depletion of N_2O_5 by photolysis during the daytime. This behaviour is in good agreement with predictions, and represents an important validation of current photochemical models of the stratosphere.

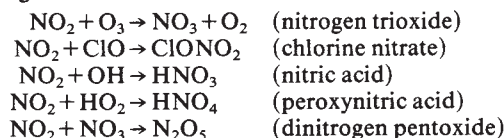
In the 1970s it was recognized that anthropogenic gases were the source of radicals, for example NO, Cl, OH, represented by the symbol X in the following equations, that could destroy ozone by the catalytic cycle



whose net result is:



The catalytic cycle involving NO and NO_2 is thought to be especially efficient, with each NO radical predicted to destroy 300 ozone molecules before being trapped by one of the five following reactions:

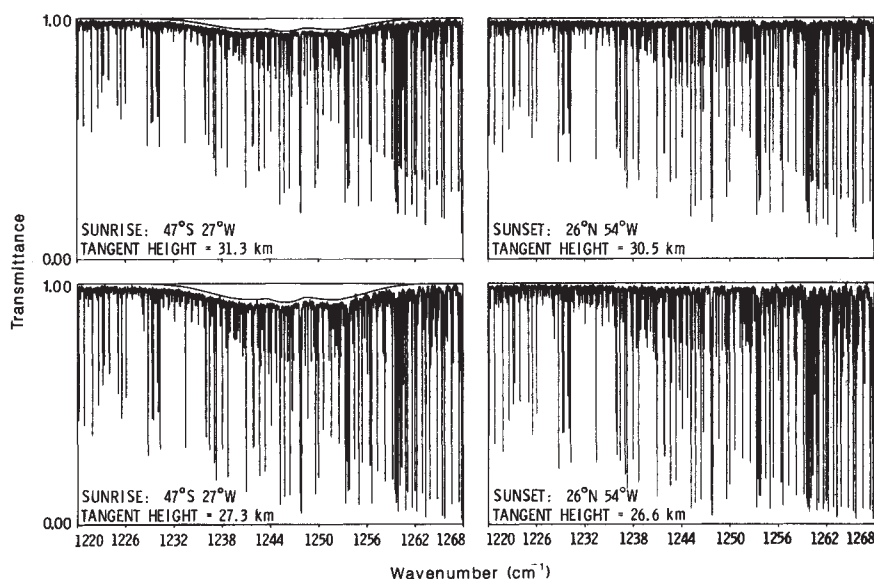


Like NO and NO_2 , these five products are thought to be present only in parts per 10^9 and, apart from nitric acid, have not yet been detected unambiguously. Identification and measurement of these products is crucial to the confirmation of photochemical theories and hence the accurate evaluation of trends in ozone amounts.

Roscoe² measured the sunrise–sunset difference in the total thermal emission over the 1,230–1,250 cm^{-1} spectral region using a balloon-borne filter radiometer. However, this was not considered an unequivocal detection because Roscoe lacked the spectral resolution necessary to show that the diurnal variation was associated with the N_2O_5 absorption band.

The ATMOS instrument consisted of a suntracker and telescope which accepted a 2-mrad field-of-view from the centre of the solar disk, a double-passed Michelson interferometer, and a HgCdTe detector cooled to 77K. Measurements were made

Fig. 1 Observed spectral transmittance of the atmosphere.



during the periods when the spacecraft was entering or emerging from the Earth's shadow. The solar radiation measured in successive one million point interferograms (2.2 seconds apart) had therefore passed through the Earth's atmosphere at different altitudes. After Fourier transformation, these interferograms yielded 0.015 cm^{-1} resolution spectra of the entire mid-infrared region at vertical intervals of 4 km. The high spectral resolution allowed the spectral lines of the interfering gases to be distinguished from the broad underlying absorption between $1,230$ and $1,260\text{ cm}^{-1}$, permitting an unambiguous identification of the ν_{12} band (symmetric NO_2 stretch³) of N_2O_5 to be made. The stronger, overlapping ν_1 and ν_{11} bands (antisymmetric NO_2 stretch³) were also evident between $1,680$ and $1,760\text{ cm}^{-1}$, but the heavy interference from HNO_3 spectral lines made their identification less clear-cut than that of the ν_{12} band.

In Fig. 1, spectra measured from an orbital altitude of 350 km on 1 May 1985 are presented. The sunrise was observed at $47^\circ\text{S } 27^\circ\text{W}$ and the sunset at $26^\circ\text{N } 54^\circ\text{W}$. A broad featureless absorption band underlies the $1,230$ – $1,260\text{ cm}^{-1}$ region of the sunrise spectra, but not the sunset spectra. To confirm that this absorption band corresponded to the ν_{12} band of N_2O_5 , we computed the absorption of N_2O_5 alone for the same geometry as the observed spectra. Climatological temperature and pressure profiles were assumed, together with the N_2O_5 volume mixing ratio profile shown in Fig. 2. The result, the smooth curves superimposed on the sunrise spectra, completely account for the observed sunrise–sunset differences, supporting the thesis that the broad absorption band in the sunrise spectra is due to N_2O_5 .

The spectral line parameters of the N_2O_5 absorption bands are not yet known. Because of the high moment of inertia of the N_2O_5 molecule, its rotational energy levels are closely spaced, giving rise to vibration-rotation bands containing large numbers of densely packed spectral lines, which are not resolved⁴ even at a spectral resolution of 0.02 cm^{-1} and a pressure of 0.15 torr. It can be estimated that a full spectral characterization of the ν_{12} band and its strongest hot bands would yield over 80,000 spectral lines of significant strength, which would render line-by-line calculations of spectra tedious. We therefore sought to represent the gross features of the shape of the ν_{12} band (and its temperature dependence) with a simple empirical model. This was done by treating the molecule as linear with a vibrational constant $B = 0.1\text{ cm}^{-1}$. Each spectral line was assigned a Lorentz width of 25 cm^{-1} , and the strengths of the Q-branch lines were reduced to only 0.3 of those of the P or R branches. The spectral line positions were adjusted until the

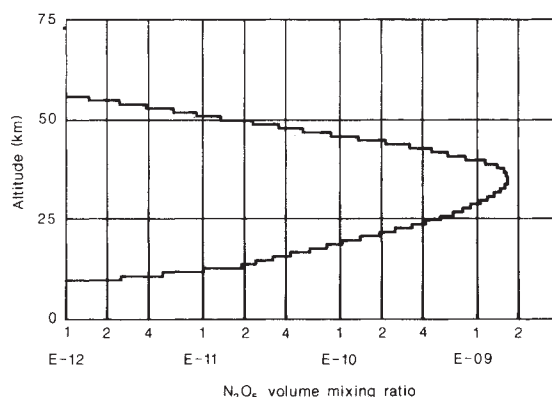


Fig. 2 Volume mixing ratio profile of N_2O_5 .

band shape at room temperature matched the laboratory measurement of Murcray *et al.*⁴. The strengths of the resulting 360 spectral lines were then scaled to give an integrated band intensity of $4.32 \times 10^{-17}\text{ cm}^{-1}/(\text{molecules cm}^{-2})$. This figure was derived from absorption cross-section measurements made by Connell⁵, which agree well with those of Graham and Johnston⁶. Recent measurements by Perner *et al.*⁷ and Cantrell *et al.*⁸ also support Connell's value. However, it should be pointed out that this value of the integrated band intensity is in disagreement with the $2.36 \times 10^{-17}\text{ cm}^{-1}/(\text{molecules cm}^{-2})$ ($581\text{ cm}^{-1}/(\text{atm-cm})$ at 298 K) measured by Lovejoy *et al.*⁹. To resolve this discrepancy, further measurements are required, preferably at stratospheric temperatures.

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Synthetic organic chemicals in the deep Sargasso Sea

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The flux of chlorinated synthetic organic chemicals to the open ocean is important both to organic geochemistry and for environmental health. We have studied over a 2-yr period the deposition of polychlorinated biphenyls (PCBs), chlordanes and dieldrin by means of a sediment trap in the Sargasso Sea. We now report that the fluxes of these materials to the deep North Atlantic Ocean were $1.6 \mu\text{g m}^{-2} \text{yr}^{-1}$, $0.02 \mu\text{g m}^{-2} \text{yr}^{-1}$ and $0.04 \mu\text{g m}^{-2} \text{yr}^{-1}$, respectively between 1978 and 1980.

Samples were collected in a 1.5 m^2 cross-section sediment trap¹ moored 3,200 m below the surface and 1,000 m above the sea floor at $32^\circ 05' \text{ N}$, $64^\circ 15' \text{ W}$, $\sim 45 \text{ km}$ south-east of Bermuda. The amounts of material collected in the trap vary with the annual cycle of productivity in surface waters². We have analysed samples collected between 1978 and 1980 over nine intervals of ~ 60 days for PCBs, chlordanes (the sum of *cis*- and *trans*-isomers) and dieldrin. Chlordane and dieldrin are among the most persistent chlorinated pesticides³, while some of the PCBs are resistant to degradation and ubiquitous in the marine environment⁴. All three classes of compounds have been found in the North Atlantic atmosphere⁵; the North Atlantic Ocean may be a sink for 50–80% of PCBs in the environment⁴.

The size fraction analysed ($<125 \mu\text{m}$) accounts for 75–90% of the mass collected by the deep trap¹. The daily flux of total PCBs ranges from 1.2 to 10.8 ng m^{-2} (Table 1), corresponding to a mean yearly flux of $1.6 \mu\text{g m}^{-2}$; the concentration on the particles ranges from 50 to $350 \text{ ng per g dry weight}$, or a mean of $\sim 150 \text{ ng per g}$. If all PCBs are contained in the $<125\text{-}\mu\text{m}$ fraction, the average concentration is 110 ng per g of total particulate matter collected.

The fluxes of chlordane and dieldrin average $58 \pm 59 \text{ pg m}^{-2} \text{day}^{-1}$ ($21.4 \text{ ng m}^{-2} \text{yr}^{-1}$) and $114 \pm 105 \text{ pg m}^{-2} \text{day}^{-1}$ ($38.4 \text{ ng m}^{-2} \text{yr}^{-1}$), respectively. The PCBs correlate with organic carbon ($r=0.81$, $n=8$) (Fig. 1), as is the case with all other constituents of this sediment-trap material^{2,6,7}. Chlordane and dieldrin also correlate with organic carbon ($r=0.72$, $r=0.74$ respectively, $n=7$). The concentration of chlorinated hydrocarbons in sample no. 5 was small compared with that in the other samples while the total mass and the carbon flux was the greatest², but we cannot tell from our limited data-set whether this total exceptionally large mass flux is the explanation. However, the relationships between the individual compounds correlate well (PCB/dieldrin, $r=0.98$, $n=7$; PCB/chlordane, $r=0.90$, $n=7$; dieldrin/chlordane, $r=0.90$, $n=7$). The small sample size means the analytical variability could only be tested on one sample (no. 4) by sub-sampling and independent analysis. The standard deviation of the replicate was 18%. Analytical blanks were very low as we used very small volumes of solvents. However, contamination from the sediment trap may have occurred as a satisfactory sampling blank could not be determined. We would expect to find components of the atmosphere over the Sargasso Sea^{8,9} in sea water¹⁰ and in deep-ocean sediments¹¹ in the trap, but not significant contamination by pesticides, chlordane and dieldrin. The presence of these organic compounds is, therefore, not caused by sampling or analytical errors.

Dissolved PCB concentrations in surface waters were measured 10 km north of the trap site in January 1980 (ref. 10)

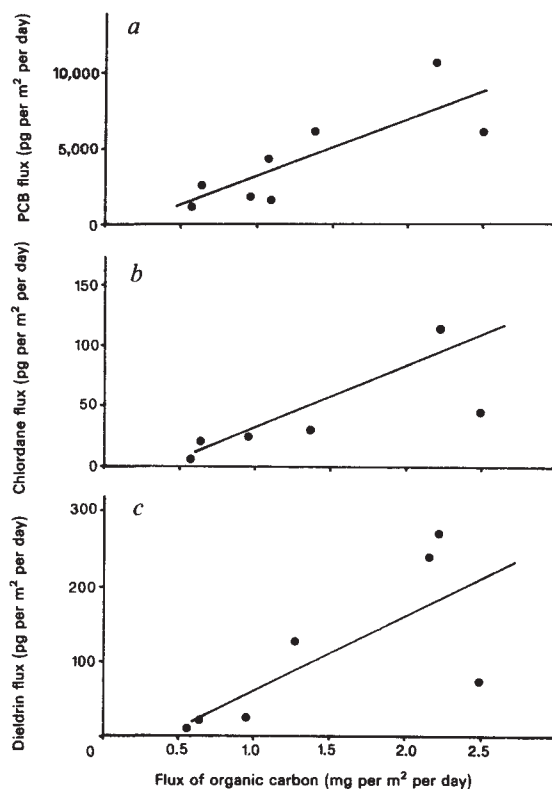


Fig. 1 a, Relationship of PCB flux to organic carbon flux. Regression coefficient $r=0.83$ ($n=8$). b, Chlordane to organic carbon: $r=0.72$, $n=7$. c, Dieldrin to organic carbon: $r=0.74$, $n=7$.

at $\sim 100 \text{ pg total PCB l}^{-1}$. The dissolved and particulate PCBs of an oligotrophic area of the Pacific Ocean have similar primary productivity and dissolved concentrations as in the water over our trap¹². Tanabe and Tatsukawa¹² found that $\sim 7\text{--}49\%$ of the total PCB found was present on glass-fibre filters although it was not known how much was desorbed from the filter. However, PCBs have low solubility in water and high octanol/water partition coefficients, so they are readily adsorbed to suspended solids, especially those high in organic carbon¹³. In contrast, $<0.5\%$ of the α and γ isomers of hexachlorocyclohexane (HCH) were adsorbed on the filters. Lindane, the γ isomer of HCH and a widely used pesticide, is far more water-soluble ($\sim 6.1 \text{ p.p.m.}$ 25°C in NaCl; ref. 14) than PCBs and chlordane and is a component of North Atlantic rain water (A.H.K., in preparation) and this is presumably why we did not detect lindane in our trap, although it may have a residence time of 10 yr in the open ocean¹². We also did not detect α -HCH on the intercepted particles.

We can compare our flux data with the more accurately known atmospheric input data^{4,5,9,15}. One can predict atmospheric vapour deposition using the average concentration of the compound in the atmosphere and its deposition velocity (v_d). Although v_d is difficult to measure for the open ocean, 0.1 cm s^{-1} seems to be the best estimate⁵. We assume the atmospheric concentration of PCBs over the open North Atlantic Ocean to be $50\text{--}100 \text{ pg m}^{-3}$ (refs 5, 9), which gives a deposition to the ocean surface of $1.6\text{--}3.1 \mu\text{g m}^{-2} \text{yr}^{-1}$. Rainwater and dry particle deposition will remove some of this material from the atmosphere, but this process should be more effective in coastal areas than the open ocean.

Based on concentrations of PCBs in a few sediment cores taken near our trap, a similar flux of $1.6 \mu\text{g m}^{-2} \text{yr}^{-1}$ was derived¹¹. A sediment trap deployment near the Tongue of the Ocean (Bahamas) yielded a flux of PCBs of $4.9 \mu\text{g m}^{-2} \text{yr}^{-1}$; this trap was only 100 m off the bottom at 2,050 m (ref. 11).

Table 1 Flux of organochlorines at 3,200 m

Sample no.	Sampling period	Duration (days)	Flux (pg m ⁻² day ⁻¹)			Flux (mg m ⁻² day ⁻¹) Organic carbon
			PCB	Chlordane	Dieldrin	
1	4 April–7 June 1978	62	6,200	31	127	1.38
2	7 June–8 August 1978	62	4,400	—	—	1.08
3	9 August–10 October 1978	62	1,600	—	—	1.10
4	12 December 1978–14 February 1979	64	10,800	165	240	2.18
5	24 March–31 May 1979	68	6,200	46	77	2.50
6	31 May–30 July 1979	60	1,900	24	25	0.96
7	10 October–3 December 1979	52	1,200	6	10	0.58
8	6 February–7 April 1980	63	—	114	274	2.22
9	15 April–17 June 1980	61	2,600	21	25	0.64
	Mean daily flux at 3,200 m (pg m ⁻² day ⁻¹)		4,362 ± 3273	58 ± 59	114 ± 105	
	Mean yearly flux (ng m ⁻² yr ⁻¹)		1,590	21	38	

All values are expressed on a per square metre basis. After recovery, the samples were collected from the cup of the trap and wet-sieved into five size fractions². The fractions <35 µm and 35–125 µm were pooled and sub-sampled for trace organic analysis. Samples were dried at 60 °C. Sub-samples ranged from 80 to 100 mg and were extracted five times with 4-cm³ aliquots of dichloromethane after a 15-min sonication and phase separation. These extracts were evaporated, taken up in hexane, separated into two fractions using Florisil adsorption chromatography⁸ and analysed by temperature-programmed electron capture gas chromatography using splitless techniques¹⁹. A 25 m × 0.2 mm SE-54 column was used for separation. Confirmation of PCBs was carried out by gas chromatographic analysis before and after treatment with 7% fuming sulphuric acid¹⁵ and identification of retention times of individual components compared with known standards¹⁹. Additional confirmation was carried out by selective ion monitoring gas chromatography/mass spectrometry using a Hewlett-Packard mass selective detector. Response factors were determined for these individual PCBs and quantification was carried out expressing the results as total PCB⁸ rather than in terms of equivalents of commercial chemical mixtures. Dieldrin and chlordane (α and γ isomers) have been quantified using authentic standards by response factors on the ECD GC. For the present purposes both chlordane isomers have been summed.

Table 2 Individual chlorobiphenyl congener particle flux in <125 µm fraction of sediment trap material at 3,200 m

IUPAC no./name of chlorobiphenyl	PCB mix	Flux (pg m ⁻² day ⁻¹)						Flux (ng m ⁻² yr ⁻¹)	
		June 1978	February 1979	March 1979	July 1979	December 1979	August 1980	Mean ± s.d.	Total flux
26 2,3',5'-tri-	a, b*	56	91	99	20	6	38	52 ± 38	19
28 2,4,4'-tri-	a, b	83	14	94	97	25	21	52 ± 44	19
44 2,2',3,5'-tetra-	a, b, c, d*	64	22	187	92	17	19	67 ± 66	24
49 2,2',4,5'-tetra-	a, b, c	—	201	133	61	17	49	92 ± 74	34
52 2,2',5,5'-tetra-	a, b, c, d	144	586	444	108	47	71	233 ± 225	85
101 2,2',4,5,5'-penta-	a, b, c, d	111	288	181	56	55	89	130 ± 90	47
118 2,3',4,4',5-penta-	a, b, c, d	188	470	340	144	117	151	235 ± 140	85
128 2,2',3,3',4,4'-hexa-	c, d	2	24	23	7	7	0	11 ± 10	4
153 2,2',4,4',5,5'-hexa-	a*, b*, c, d	58	103	79	31	25	31	55 ± 35	8
174 2,2',3,3',4,5,6'-hepta-	c*, d	7	33	34	14	7	14	18 ± 12	6
180 2,2',3,4,4',5,5'-hepta-	c, d	25	61	56	11	21	9	31 ± 23	11
201 2,2',3,3',4',5,5',6-octa-	d	—	26	29	17	10	14	19 ± 11	6

These individual congeners were chosen according to their distribution in most of the samples. They are compounds that are well separated by our chromatographic techniques into single peaks and that have been identified as individual compounds by gas chromatography/mass spectrometry¹⁸. The only exception are peaks 28 and 153 which can sometimes co-elute with other peaks. A detailed analysis of the commercial PCB mixtures of Clophen has been carried out¹⁸. A series of products with different degrees of chlorination was analysed (30, 40, 50 and 60% chlorine). Compounds such as 44, 52, 101 and 118 were present in all PCB mixtures of differing chlorination. No. 49 is present in three of the four mixtures. The others are only present in two of the four products or were minor constituents in all but one mixture. a, Clophen A30; b, Clophen A40; c, Clophen A50; d, Clophen A60.

* Minor constituent in mixture.

Fluxes of PCBs measured in the Mediterranean were ~20 times greater¹⁶ and in the Baltic Sea, in 20 m of water, 80 times greater than our Sargasso Sea values¹⁷. Considering the difference in magnitude of the source functions in the Mediterranean and Baltic compared with the open ocean, we consider this divergence reasonable.

Conventional analyses of PCBs are reported as the concentration in terms of commercial mixtures (Clophen or Aroclor equivalents). But this does not give information on sources, sinks and behaviour of individual PCB congeners in the environment, as each congener may behave differently^{4,18}. Analysis by capillary gas chromatography is the preferred method, with calibration against accurate standards.

We have identified some individual congeners in the trap, and have quantified those for which we have standards and the

correct identification. Table 2 shows that many of these individual components have different sources from various industrial mixtures and have undergone different biogeochemical modifications. The three components with the greatest flux are 52, 101 and 118 which are present in all of the most widely used commercial mixtures¹⁸. The other individual components have a generally lower concentration relating to the number of technical mixtures in which each compound appears.

Our data indicate that PCBs were major contaminants on particles in the open ocean up till 1980, although production in the United States ceased in 1977. The analysis of the individual components of these synthetic chemicals is important as their absence or presence in parts of the marine environment provides an insight into biogeochemical processes in the ocean.

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A lagrangian mixed layer model of Atlantic 18 °C water formation

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One of the most striking features of the upper North Atlantic Ocean is an extensive layer of water with temperature close to 18 °C and salinity close to 36.5‰ (ref. 1). This 18 °C water is formed by winter convection in the Sargasso sea^{2,3}, but aspects of the annual rate of 18 °C water formation remain obscure⁴. We have simulated this water mass formation by integrating a one-dimensional model along a 4-yr trajectory of a water column circulating around the Sargasso Sea. Winter convection is deep (≥ 200 m) in regions where the ocean suffers a net annual heat loss to the atmosphere, and shallow (≤ 100 m) where the ocean gains heat each year. The origin of the thermostat (nearly isothermal layer) is a thick layer of nearly homogeneous water subducted beneath the seasonal boundary layer in the year that the water column passes through the line dividing annual cooling from annual heating. We estimate the annual production of 18 °C water to be 446,000 km³ yr⁻¹. Downstream, more stratified central water is formed each year at a rate that depends more on Ekman pumping (wind-forced convergence) than on the decreasing depth of winter convection.

On 29 March 1873 the water temperature measurement for HMS *Challenger* station no. 28 (24°39' N, 65°26' W) was repeated because the water appeared to be anomalously warm at 200 fathoms. It was suspected that the thermometers were faulty, but replacements gave the same values^{5,6}. That was the first encounter with 18 °C water, which has since been mapped over a large area of the North Atlantic⁴. It is now recognized to be typical of a class of water masses called mode waters,

formed by deep convection in winter⁷⁻¹¹. The depth of convection is sensitive to small changes in the net annual surface buoyancy flux, B (refs 12, 13). The interannual variation of winter climate in the Sargasso Sea is believed to vary the annual production of 18 °C water¹⁴⁻¹⁶. Attempts have been made to estimate this variation in 18 °C and other mode water^{17,18}, which may be a significant factor in the ventilation of climatically-induced anomalies into the permanent thermocline¹⁹. Thermocline ventilation is an important factor in the oceanic influence on climate, and will have to be accurately simulated in future climate prediction models²⁰. The rate of mode water formation depends on a complicated non-linear interaction between the seasonal boundary layer and the gyre circulation²¹. It can be computed with general circulation models (GCMs)^{22,23} but the results may be in error because of simplifications in their treatment of the complex process. The model presented here clarifies issues that will arise in redesigning GCMs to improve simulation of mode water formation.

Our mixed layer model pays particular attention to the treatment of convection²⁴. It was tested in the eulerian mode, by integrating it with a time series of surface fluxes at a location where $B = 0$. Such eulerian integrations have successfully simulated the observed variation of upper ocean structure at sites such as Ocean Weather Station (OWS) 'P', where advectively-induced changes are small²⁵. But neglecting advection poses a severe problem for modelling the seasonal boundary layer at most sites. The solution of that problem is a prerequisite for modelling water mass formation. It is particularly severe in the North Atlantic where the advective heat flux divergence is large²⁶. Stommel²⁷ investigated the dynamics of advective heating at OWS 'J' using IGY hydrographic data. In general, however, the database is inadequate to diagnose the circulation sufficiently accurately for advective correction terms in eulerian one-dimensional models of the mixed layer.

Woods²¹ proposed a lagrangian solution in which the model is integrated at successive positions along a trajectory derived from observations or a circulation model. Lagrangian analysis of climatological maps of mixed layer depth and density showed the potential of the technique²¹. The thickness H of the water subducted in 1 yr along such a trajectory is given by the water mass equation, $H = E + D$, where E is the annual vertical displacement due to Ekman pumping and D is the decrease in the annual maximum depth of the mixed layer between successive years. Central water subduction has traditionally²⁸ been related to E , but according to the new theory D is also important²⁹. The theory supported the established view that the rate of mode water formation is controlled by convection, that is $D \gg E$. We now report the first test of that theory by lagrangian integration of a mixed-layer model.

The model was integrated with 12-hour time steps using solar elevation parameterization²⁴. The surface fluxes and Ekman pumping were taken from refs 30, 31 for successive daily positions along a trajectory (Fig. 1) derived from the Princeton GCM²⁶. Integrations were started on day 90 using climatological initial conditions³². The model neglected advective changes in the mixed layer temperature and salinity due to Ekman transport (which preliminary calculations showed to be small) and due to the vertical shear of the geostrophic flow. The model was integrated 12 times with initial starting positions displaced upstream to make the integration pass a reference point (the 500-km mark in Fig. 1) at 1-month intervals.

Figure 1 shows the variation of (daily maximum) mixed layer depth and the depths of selected isopycnals for the run that passed through the reference point on day 90. The annual maximum mixed-layer depth is reached on a day near the spring equinox. Its value increases slightly in the first year because $B \leq 0$, but then decreases as the integration enters the zone of net annual buoyancy gain. The large value of H in the year that the integration passes through the $B = 0$ line indicates the subduction of a thick layer of water below the seasonal boundary

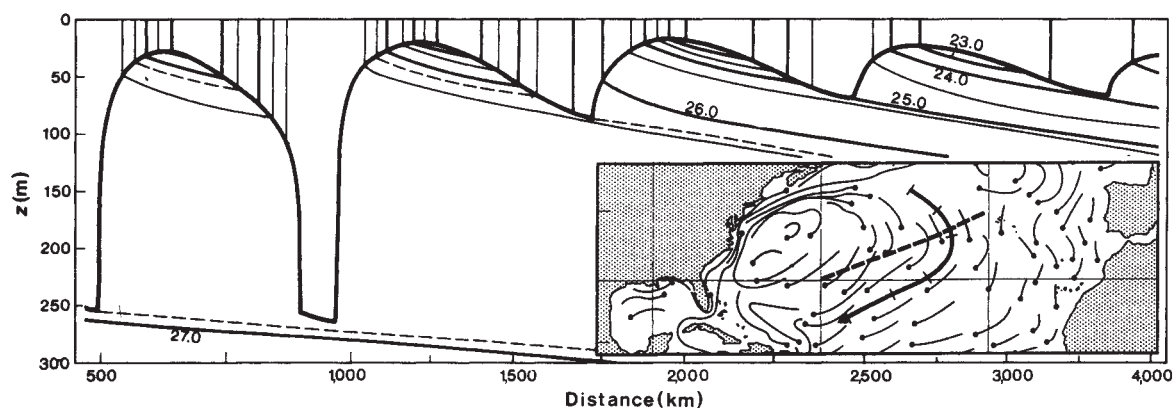


Fig. 1 Variation of mixed-layer depth and the depths of selected isopycnals (density (σ_t) values at 0.5 kg m^{-3} intervals, plus 26.75 and 26.25 kg m^{-3} dashed) for a lagrangian integration of a mixed-layer model²⁴ along the trajectory shown in the inset map. The integration started on day 90 at the reference position 500 km from the start of the trajectory. Inset, trajectory used in the lagrangian integration with 1-yr tick marks (bold line); $B=0$ according to ref. 30 (dashed bold line); annual particle displacements at a depth of 159 m from ref. 26 (fine lines).

layer. The subduction is effected rapidly as the mixed-layer depth decreases in the spring. The mixed-layer density decreases at the same time, so the subducted water is statically stable. However, the total change in density (and temperature) is small, so the subducted water has the characteristic pycnostad (thermostad) of a mode water. In this case, the mode water has a temperature of 17.0°C , and a salinity of 36.5% , which lies in the range for class II 18°C water¹.

After the mode water has been formed, the model predicts that D becomes much smaller, but remains positive as long as B is positive. The magnitude of D becomes smaller than E , showing that Ekman pumping becomes relatively more important in determining the annual production of central water downstream of the subduction of mode water. Figure 2 shows the variation of D and E along the trajectory, derived from the 12 integrations with phase lags of 1–12 months. The mode water is implanted along a section straddling the $B=0$ line. The length of the sector equals the distance the water moves in that year. The break in the D curve near $1,000 \text{ km}$ arises from the differences between integrations started at 40 and 500 km . It represents a 25% range of uncertainty in the method. The difference between the curves for integration with and without Ekman pumping is due to the Ekman convergence of heat (which would, in part, be compensated by baroclinic geostrophic heat flux divergence if that were included). It does not significantly change the annual rate of mode water mass production.

The map of North Atlantic mixed-layer depth for April, when 18°C water is being formed, shows a line of maximum D close to the $B=0$ line (Fig. 3). The annual production of 18°C water computed from the sum of the products of the length, spacing and D values³² from the 10 vectors west of the Azores in Fig. 3 is $446,000 \text{ km}^3 \text{ yr}^{-1}$; close to Worthington's³³ value of $230,000 \text{ km}^3 \text{ yr}^{-1}$ based on the surface heat flux in the Sargasso Sea. Our value should, however, be treated with caution, given the uncertainties in the vectors and the map of mixed layer depth.

The volume of 18°C water is $1,672,000 \text{ km}^3$ (ref. 4), so our formation rate gives a mean renewal time of 4 yr, and Worthington's rate gives 7 yr. Independent estimates based on radioactive tracers^{34,35} give values $<10 \text{ yr}$, but not precise enough to discriminate between Worthington's value and ours.

In principle, the mass and temperature–salinity (T–S) properties of mode water formation can also be computed by integrating the model along a set of trajectories that cross the $B=0$ line. In practice, the calculation would not be more reliable because of: (1) uncertainties in the trajectories derived from GCM simulations²⁰; (2) neglect of vertical shear in the seasonal thermocline, which may include a significant component across the chosen trajectory³⁶; (3) errors of up to $\pm 50 \text{ W m}^{-2}$ in the climatological estimates of surface heat flux³⁷, making the location of the $B=0$ line uncertain to about $\pm 500 \text{ km}$; and (4)

imperfections in the mixed layer model. These sources of error combine to give a growing deviation from the observed mixed-layer depth and temperature³². Furthermore, the thickness of the mode water subducted in the year that the water passes the $B=0$ line depends on the assumed initial value for mixed-layer depth, taken from observations³², which depend critically on the method of diagnosis²¹.

Despite these limitations to quantitative application, the lagrangian mixed-layer model yields two conclusions. The first is that, in common with other mode waters, 18°C water is formed in a band straddling the $B=0$ line, with a downstream width equal to the distance water travels in that year. The second is that the annual rate of water mass subduction depends on both D and E , with D dominating mode water, and E dominating

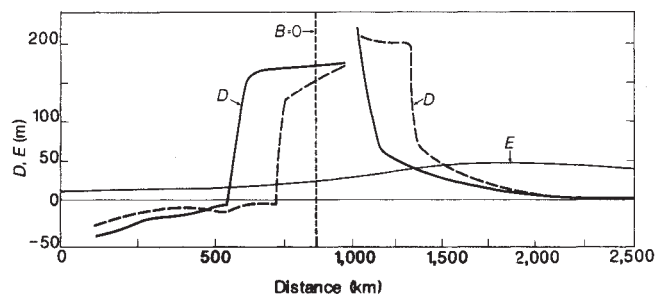


Fig. 2 Variation of D and E along the trajectory in Fig. 1 (inset) based on a set of lagrangian integrations with monthly phase lags. The mode water is formed where D has a large value. Its downstream extent equals the distance the integration advances in that year. Solid line, with Ekman pumping; dashed line, without Ekman pumping. The discontinuities in the two lines near $1,000 \text{ km}$ represent the different values of D computed from starting points at 40 and 500 km respectively. They indicate a 25% range of uncertainty in D .

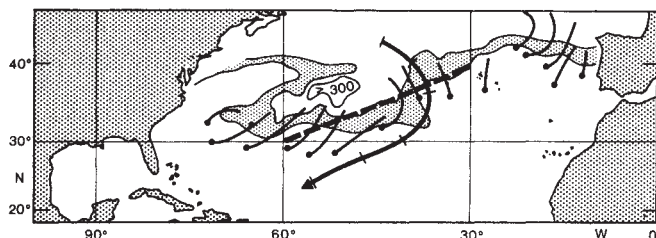


Fig. 3 The formation zone of mode water is indicated by a stippled band between the mixed layer depths of 120 and 200 m for the month of April³², when mode water is being subducted. The dashed line indicates $B=0$ according to ref. 30. Selected annual displacement lines from Fig. 1 show where mode water is formed. East of the Azores it is the North Atlantic sub-polar mode water¹⁹; west of the Azores it is 18°C water.

central water formed downstream of the mode water. The lagrangian method can be used generally to analyse water mass formation.

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Isotope anomalies induced in laboratory distillation

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We have carried out high-temperature distillation experiments at 5×10^{-5} torr on terrestrial pyroxene crystals and measured ^{24}Mg , ^{25}Mg and ^{26}Mg simultaneously using a 61-cm multi-cup mass spectrometer. The raw data were corrected for isotope fractionation using the power law and the Rayleigh law. Regardless of the correction procedure, the sample residues are depleted in ^{26}Mg and the condensates are enriched in ^{26}Mg , relative to non-distilled standards. These effects mimic the 'isotope anomalies' in Mg observed in Allende coarse- and fine-grained inclusions, previously attributed to nucleosynthetic processes involving ^{26}Al .

We subjected pyroxene crystals from a sample of eclogite (Yakutia, Soviet Union) to distillation. Each sample, weighing ~50 mg, was placed in a carbon boat and heated with a radio-frequency induction coil in a bell-jar in a vacuum ($\sim 5 \times$

Table 1 Electron-microprobe analyses of pyroxenes before and after distillation

	Original (light green)	First residue (transparent)	Second residue (blue sphere)*
SiO_2	54.5	36.5	<0.1
TiO_2	0.2	<0.1	0.6
Al_2O_3	2.9	10.7	76.5
Cr_2O_3	1.0	<0.1	<0.1
FeO	1.2	<0.1	<0.1
MgO	16.5	12.5	<0.1
CaO	23.4	40.3	22.4
Na_2O	0.5	<0.1	<0.1
Total	100.2	100.0	99.5

Wavelength-dispersive analyses in weight per cent.

* Further vaporization of the first residue produced an opaque milky-white sphere. Treatment with HF and HNO_3 left a residual bright blue 2-mm diameter sphere, analysis of which showed it to be composed of CaAl_4O_7 with a small amount of Ti. It is interesting that thermodynamic calculations predict hibonite ($\text{CaAl}_{12}\text{O}_{19}$) and CaAl_4O_7 to be the only two stable calcium aluminates during condensation¹⁴; it might therefore be expected that they would both appear as evaporation residues.

10^{-5} torr). After melting, the samples coalesced into small spheres, spinning rapidly and jumping in the carbon boat, and they began to vaporize at $\sim 2,150^\circ\text{C}$. As the vaporization proceeded the sample changed in colour from light green to completely transparent. Chemical analysis of the residue at this stage showed that most of the iron in the sample had already vaporized. Part of the vaporized material was collected on 25-mm diameter Teflon disks placed ~8 cm above the carbon boat. Nine samples were subjected to varying degrees of distillation for up to 30 min, and the weight of each sample was recorded before and after evaporation. Typical electron-probe analyses of the pyroxenes before and after distillation are shown in Table 1.

The evaporation residues and condensates were next dissolved in a mixture of HF and HNO_3 . Small aliquots of these samples were directly loaded on V-shaped Re filaments for Mg isotope analysis. The intermediate step of chemical separation is avoided in the direct-loading technique. There are no interfering isobar species in the Mg region of the mass spectrum. In contrast to other mass spectrometers¹, the ANU 61-cm multi-cup instrument used does not suffer from interferences caused by scattered beams near the Mg isotopes. (The details and the operating characteristics of this instrument in relation to the determination of Mg isotope abundances have been described previously².) For each distillation sample, the raw data consist of a series of sets of simultaneous values of the intensities of each Mg isotope. Data collection and processing procedures have been described previously^{2,3}. For each set a fractionation-corrected value of the ^{26}Mg -to- ^{24}Mg ratio, $(^{26}\text{Mg}/^{24}\text{Mg})^c$, was obtained by applying a specific fractionation law and normalizing the $(^{25}\text{Mg}/^{24}\text{Mg})$ ratio to the National Bureau of Standards standard value of 0.12663 (ref. 4). The fractionation-corrected value for the sample was then compared with the fractionation-corrected value obtained, using identical procedures, for a set of non-distilled standards $((^{26}\text{Mg}/^{24}\text{Mg})^c_s)$. The difference between these two values is represented, in parts per mil (‰) by the quantity δ , defined as

$$\delta(^{26}\text{Mg}) = [(^{26}\text{Mg}/^{24}\text{Mg})^c / (^{26}\text{Mg}/^{24}\text{Mg})^c_s - 1] \times 1,000$$

and may be regarded as a measure of the inadequacy of the specific fractionation law used to account for the fractionation processes involved in the distillation. Non-zero values of residual fractionation δ (isotope anomalies) obtained in the past for meteoritic samples have been attributed to nucleosynthetic effects⁵ involving ^{26}Al , sometimes termed nonlinear effects⁶.

The Mg isotope analyses for the standards and for the residues and condensates are summarized in Table 2 and Fig. 1. The data

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Table 2 Distillation-induced fractionation and residual isotope effects in Mg (± 2 s.d.)

Sample and mass loss (%)	Un-normalized raw ratios*		Fractionation†	$\delta^{26}\text{Mg}\ddagger$ (power law) ± 2 s.d.	$\delta^{26}\text{Mg}\S$ (Rayleigh law) ± 2 s.d.
	$(^{25}\text{Mg}/^{24}\text{Mg})_{\text{meas}}$	$(^{26}\text{Mg}/^{24}\text{Mg})_{\text{meas}}$	$\Delta(^{25}\text{Mg}/^{24}\text{Mg})\% \text{ AMU}^{-1}$		
1 Condensate	0.123684	0.134678	-1.4	$+0.38 \pm 0.16$	$+0.36 \pm 0.16$
2 Residue (23)	0.124263	0.135884	+3.3	-0.02 ± 0.07	$+0.05 \pm 0.06$
Condensate	0.122352	0.132055	-12.2	$+2.33 \pm 0.12$	$+2.09 \pm 0.12$
3 Residue (42.6)	0.124930	0.137255	+8.7	-0.67 ± 0.06	-0.50 ± 0.07
Condensate	0.122998	0.133359	-6.9	$+1.64 \pm 0.08$	$+1.50 \pm 0.08$
4 Residue (59)	0.127204	0.142233	+27.0	-1.04 ± 0.08	-0.51 ± 0.07
Condensate	0.123688	0.134734	-1.4	$+0.74 \pm 0.05$	$+0.72 \pm 0.05$
5 Residue (32.7)	0.124327	0.135996	+3.8	-0.23 ± 0.06	-0.15 ± 0.06
Condensate	0.122176	0.131616	-13.6	$+1.87 \pm 0.06$	$+1.60 \pm 0.07$
CL condensate	0.123111	0.133525	-6.1	$+1.06 \pm 0.08$	$+0.94 \pm 0.07$
BG residue (51)	0.126094	0.139846	+18.1	-0.48 ± 0.16	-0.12 ± 0.16
6 First condensate	0.122136	0.131436	-13.9	$+1.16 \pm 0.06$	$+0.88 \pm 0.05$
Second condensate	0.124363	0.136154	+4.1	$+0.36 \pm 0.08$	$+0.44 \pm 0.07$
7 First condensate	0.122107	0.131327	-14.1	$+0.80 \pm 0.23$	$+0.52 \pm 0.22$
Second condensate	0.124150	0.135509	+2.4	-0.97 ± 0.06	-0.92 ± 0.06
Standards					
1	0.123858	0.134987			
2	0.123854	0.135005			
3	0.123876	0.135043			
4	0.123834	0.134953			
5	0.123863	0.135022			

Atomic masses used throughout.

* Directly measured, unnormalized raw ratios. Typical errors range from 2 to 4 in the last digit for $^{25}\text{Mg}/^{24}\text{Mg}$ and four to eight for $^{26}\text{Mg}/^{24}\text{Mg}$. Each number is the mean of $\sim 1,200$ ratios, obtained in ~ 1 h.

† $\Delta(^{25}\text{Mg}/^{24}\text{Mg}) = [(^{25}\text{Mg}/^{24}\text{Mg})_{\text{meas}} / (^{25}\text{Mg}/^{24}\text{Mg})_S - 1] \times 1,000$, where S denotes the grand mean value: $^{25}\text{Mg}/^{24}\text{Mg} = 0.123857$ for raw data determined from standards. Reproducibility for repeat runs is better than $\pm 1.5\%$.

‡ Per mil deviation of $^{26}\text{Mg}/^{24}\text{Mg}$ relative to 0.141102 (grand mean value for standards normalized using the power law) after correcting for fractionation using the power law^{2,3}, by normalizing $(^{25}\text{Mg}/^{24}\text{Mg})_{\text{meas}}$ to 0.12663; errors are 2 s.d. of the mean.

§ Per mil deviation of $^{26}\text{Mg}/^{24}\text{Mg}$ relative to 0.140985 (grand mean value for standards normalized using the Rayleigh law) after correcting for fractionation using the Rayleigh law^{2,3}, by normalizing $(^{25}\text{Mg}/^{24}\text{Mg})_{\text{meas}}$ to 0.12663; errors are 2 s.d. of the mean.

|| The configuration of the mass spectrometer was altered for the present experiments by substituting three Solatron voltmeters in place of the existing Keithley voltmeters. This has reduced the dead time from 70 to $< 5\%$, but has resulted in a slight change in the mean values for our standards from those measured previously. This change does not affect our conclusions in this or any previous paper.

in Table 2 show the following: (1) Significant isotope fractionation ($\Delta(^{25}\text{Mg}/^{24}\text{Mg})$) can be induced in the laboratory by distillation; (2) the evaporation residues are enriched in the heavy Mg isotopes and the evaporation condensates in the light Mg isotopes; (3) distillation induces residual offsets ($\delta^{26}\text{Mg}$) that are not accounted for by the simple fractionation-correction procedures used. Neither power-law nor Rayleigh-law corrections account for all of the observed effects. The application of the exponential fractionation law of Russell *et al.*³ produces somewhat smaller values of $\delta^{26}\text{Mg}$, but the effects remain significant; (4) the residual negative/positive effects ($\delta^{26}\text{Mg}$) are correlated with positive/negative mass fractionation in a manner identical to that found in Allende coarse- and fine-grained inclusions^{5,7}. We have plotted the variation of the residuals ($\delta^{26}\text{Mg}$) with the degree of fractionation ($\Delta(^{25}\text{Mg}/^{24}\text{Mg})$) obtained using the power-law correction (Fig. 1). Our corrected data are thus directly comparable with the meteoritic data for Mg isotopes^{5,8}, which have been almost exclusively analysed using the power law. The condensates seem to exhibit larger values of $\delta^{26}\text{Mg}$ compared with residues for a given magnitude of fractionation ($\Delta(^{25}\text{Mg}/^{24}\text{Mg})$). Although there is an approximately linear trend for both sets, there is no precise correlation with fractionation. In a closed system, consideration of mass-balance requires that the isotope effects in the residue should complement the effects in the condensate. The significant non-complementarity in the data both for fractionation ($\Delta(^{25}\text{Mg}/^{24}\text{Mg})$) and $\delta^{26}\text{Mg}$ between members of residue/condensate pairs is puzzling. Evidently, condensation was selective with an isotopically heterogeneous spatial distribution.

In two cases the condensing material was sampled in stages to reveal more of the isotope systematics in the distillation process (Table 2). Consideration of equipartition of energy

requires that the light isotopes vaporize preferentially relative to heavy ones. The residue will gradually become enriched in the heavy isotopes and the condensate in the light. However, for a finite-sized sample, the residue will eventually be so depleted in the light isotopes that the vaporizing material will pass from being enriched in the light isotopes through normal to being enriched in the heavy isotopes. This phenomenon is observed in the differentially collected condensates; in both experiments the initial condensate is relatively enriched in the light Mg isotopes whereas the final condensate is enriched in the heavy isotopes. An identical effect was observed by Russell *et al.* using calcium³. Our samples show a similar but complementary effect for the residue ($\delta^{26}\text{Mg}$) remaining after

Table 3 Complementary properties of fine- and coarse-grained Allende calcium-aluminium inclusions (CAI)

Coarse-grained CAI	Fine-grained CAI
Rim layers of fine-grained material on the outside ¹²	Composed of an aggregation of small bodies ($< 10 \mu\text{m}$) with similar rim layers on each one as in coarse-grained inclusions
Rare-earth element (REE) abundances mostly of Group I type. FUN inclusion ⁸ EK-1-4-1 is an exception with Group II-type REE	REE abundances predominantly of Group II type
Positive Mg isotope fractionation: $\Delta(^{25}\text{Mg}/^{24}\text{Mg})$ enriched in the heavy Mg isotopes	Negative Mg isotope fractionation: $\Delta(^{25}\text{Mg}/^{24}\text{Mg})$ enriched in the light Mg isotopes
Negative anomaly in $\delta^{26}\text{Mg}$	Positive anomaly in $\delta^{26}\text{Mg}$

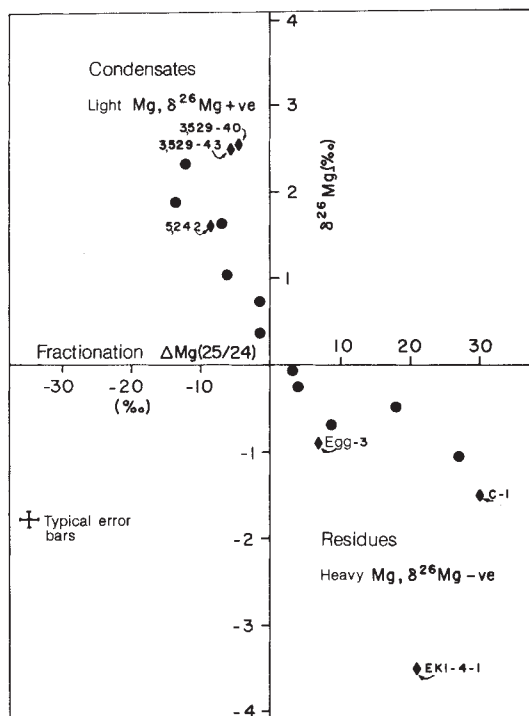


Fig. 1 Variation of $\delta^{26}\text{Mg}$ with the degree of fractionation $\Delta(^{25}\text{Mg}/^{24}\text{Mg})$ in distillation residues and condensates. The labelled data points (diamonds) represent Allende CAI with established fractionation and residual $\delta^{26}\text{Mg}$. Inclusions 3,529–40, 3,529–43 and 5,242 are fine-grained inclusions with Group II type REE pattern⁷. In contrast, inclusions Egg-3, C-1 and EK1-4-1 are coarse-grained⁵. With the exception of EK1-4-1, the CAI shown here lie within the field defined by the laboratory experiments. Note the correlation between coarse-grained CAI and evaporation residues, and between fine-grained CAI and evaporation condensates, with regard to both fractionation and residual $\delta^{26}\text{Mg}$.

the fractionation correction. Isotope analyses of the residues from the differential condensation experiments were unsuccessful. The Mg signal obtained was very low ($< 10^{-14}$ A) and could not be sustained for more than 15 min, consistent with the electron-probe analyses of such samples which show no detectable amounts ($< 0.1\%$) of MgO (Table 1).

We have included some of the data from fractionated Allende inclusions in Fig. 1^{5,9-12}, also corrected for fractionation using the power law^{2,3}. We draw attention to the complementary properties of the coarse- and fine-grained Allende inclusions (Table 3)⁷. The symmetries between the coarse- and fine-grained inclusions are replicated in the division between our distillation residues and condensates, suggesting that the coarse-grained inclusions are residues and the fine-grained inclusions are condensates. The interpretation of the excesses in the fine-grained group II aggregates could be complicated by the possible presence of ^{26}Al decay products. However, it is plausible that the observed excesses⁷ in ^{26}Mg in inclusions 3,529–40, 3,529–43 and 5,242 are wholly caused by kinetic effects and not ^{26}Al , as they lie completely within the field defined by the data for condensates. Similarly, the deficits^{8,13} in ^{26}Mg for the coarse-grained FUN⁸ inclusions C-1, EK-1-4-1 and Egg-3 are also attributable to kinetic effects rather than to a deficient ^{26}Al component (Fig. 1). Note that EK-1-4-1 seems to be distinctly shifted from the general trend defined by the data for the residues. In summary, when we apply to distillation samples the same fractionation-correction procedures that have been applied in the past to meteoritic samples, we obtain excesses and deficits in Mg isotopes similar to those reported in the meteoritic samples. These meteoritic anomalies were previously attributed to

nucleosynthetic processes. We conclude that such deductions should be made only after the effects of inadequacies in the fractionation laws used have been carefully evaluated.

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An exceptionally large late Quaternary eruption from New Zealand

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A thick, widespread volcanic ash layer sampled from marine cores in the South Pacific and sub-Antarctic, has been identified using major and rare-earth element chemistry. It is the correlative of the airfall Mount Curl Tephra, the initial eruptive phase of the voluminous Whakamaru Ignimbrite from Taupo, New Zealand. Detailed oxygen-isotope analysis on the late Quaternary section of the core from Deep Sea Drilling Project Site 594, supplemented by other data, places Mount Curl Tephra within the glacial, Isotope Stage 8 and assigns an age of 254 ± 2 kyr to the tephra. The airfall tephra covers at least 10^7 km² with a minimum volume of 700 km³. The Whakamaru Ignimbrite phase encompasses at least another 500–1,000 km³. A combined volume of $> 1,200$ km³ makes this Taupo eruption the largest in the Southern Hemisphere in late Quaternary times. Mount Curl Tephra is so widespread that the eruption possibly caused disruption to the atmosphere and climate, and material from this eruption may be recognizable in sediments 10,000 km from the source.

The dispersal of volcanic ash from recent eruptions such as Tambora (1815), Krakatau (1883), Agung (1963), Mount St Helens (1982) and El Chichón (1982) has been directly observed to circle the globe^{1–5}. Most of the ash from these eruptions has been dispersed through the atmosphere without leaving a distal deposit that would survive for any length of time in the geological record¹. In contrast, studies of large prehistoric eruptions have relied on the preserved ash to demonstrate their exceptional magnitude and the global circulation patterns responsible for their dispersal (see ref. 6). To date, such studies have dealt with tephra from equatorial or Northern Hemisphere volcanoes such as Toba, Santorini and Campanian^{7,8}. The extremely widespread dispersal of ash from a single Southern Hemisphere eruption has not been documented previously.

Table 1 Major element chemistry of glass shards (mean of *n* analyses with 1 s.d. in parentheses) from marine cores and land exposures, recalculated water-free

	RC9-110	RC9-113	RC12-215	DSDP 591	DSDP 594	Q203
SiO ₂	78.01 (0.42)	77.73 (0.28)	77.93 (0.28)	77.14 (0.21)	77.06 (0.37)	78.22 (0.32)
Al ₂ O ₃	12.40 (0.26)	12.59 (0.12)	12.26 (0.17)	12.42 (0.10)	12.47 (0.19)	12.30 (0.18)
TiO ₂	0.14 (0.05)	0.13 (0.03)	0.12 (0.03)	0.15 (0.03)	0.15 (0.03)	0.14 (0.04)
FeO	0.94 (0.18)	1.06 (0.11)	0.87 (0.11)	1.07 (0.08)	1.03 (0.09)	1.02 (0.08)
MgO	0.12 (0.04)	0.11 (0.02)	0.12 (0.02)	0.12 (0.03)	0.11 (0.03)	0.11 (0.04)
CaO	0.79 (0.07)	0.74 (0.05)	0.78 (0.05)	0.82 (0.03)	0.81 (0.04)	0.75 (0.04)
Na ₂ O	3.48 (0.24)	3.49 (0.04)	3.53 (0.14)	3.60 (0.13)	3.67 (0.13)	3.24 (0.13)
K ₂ O	4.11 (0.32)	4.15 (0.18)	4.38 (0.18)	4.46 (0.17)	4.46 (0.12)	4.21 (1.18)
Water	4.71 (0.86)	4.64 (0.85)	4.38 (1.03)	5.21 (0.45)	6.14 (0.12)	5.42 (0.67)
<i>n</i>	18	11	18	14	12	13
Age	—	0.31 s ¹¹	0.31 s ¹⁴ 0.33 g ¹⁹	0.24 ¹⁷	0.28 s ¹⁷	—
CV _m	3.02	2.74	6.31	4.48	3.65	3.01
CV _t	—	—	9.41	—	—	—
	Q585	E17-10	E33-3	E33-16	038	103
SiO ₂	77.74 (0.26)	75.38 (0.48)	76.14	62.93 (2.81)	77.75 (0.27)	78.09 (0.30)
Al ₂ O ₃	12.55 (0.27)	13.14 (0.48)	12.92	14.51 (1.08)	12.59 (0.16)	12.33 (0.21)
TiO ₂	0.12 (0.03)	0.21 (0.01)	ND	0.75 (0.13)	0.14 (0.03)	0.14 (0.03)
FeO	1.04 (0.07)	2.10 (0.12)	2.22	8.36 (0.48)	1.14 (0.06)	1.01 (0.08)
MgO	0.12 (0.04)	ND	ND	ND	0.11 (0.07)	0.12 (0.02)
CaO	0.74 (0.03)	0.37 (0.06)	0.36	1.39 (0.45)	0.79 (0.03)	0.79 (0.04)
Na ₂ O	3.42 (0.16)	4.19 (0.16)	3.87	7.22 (0.81)	3.55 (0.18)	3.30 (0.16)
K ₂ O	4.17 (0.20)	4.78 (0.13)	4.49	4.85 (0.23)	4.13 (0.15)	4.33 (0.15)
Water	3.61 (0.74)	11.21 (2.29)	7.53	1.57 (1.44)	4.95 (0.92)	4.55 (1.25)
<i>n</i>	8	6	1	11	10	132
Age	—	0.37 s ¹³	0.37 s ¹³	—	—	0.23 g ¹⁸ 0.24 z ²¹ 0.25 k ¹⁸
CV _m	4.77	—	—	—	4.52	1.00
CV _t	—	—	—	—	4.66	1.00
	122	165	166	168	207	208
SiO ₂	77.82 (0.40)	78.26 (0.14)	77.65 (0.25)	78.20 (0.32)	77.91 (0.22)	78.17 (0.29)
Al ₂ O ₃	12.58 (0.28)	12.26 (0.14)	12.41 (0.10)	12.42 (0.13)	12.31 (0.10)	12.19 (0.11)
TiO ₂	0.13 (0.04)	0.16 (0.03)	0.13 (0.02)	0.13 (0.05)	0.15 (0.03)	0.14 (0.03)
FeO	1.10 (0.09)	1.11 (0.07)	1.05 (0.07)	1.02 (0.10)	1.06 (0.07)	1.05 (0.06)
MgO	0.10 (0.09)	0.14 (0.03)	0.12 (0.01)	0.12 (0.03)	0.12 (0.02)	0.12 (0.03)
CaO	0.77 (0.04)	0.75 (0.05)	0.79 (0.05)	0.77 (0.09)	0.80 (0.04)	0.75 (0.05)
Na ₂ O	3.50 (0.09)	2.96 (0.13)	3.73 (0.11)	3.23 (0.24)	3.35 (0.26)	3.21 (0.15)
K ₂ O	4.00 (0.20)	4.37 (0.13)	4.12 (0.18)	4.11 (0.18)	4.30 (0.19)	4.37 (0.21)
Water	6.06 (1.11)	2.88 (0.46)	3.13 (0.56)	5.20 (0.90)	4.71 (0.65)	3.93 (0.86)
<i>n</i>	10	10	12	16	20	22
Age	—	—	—	0.38 g ¹⁹ 0.37 z ⁴³	—	0.33 z ⁴²
CV _m	5.44	6.07	4.31	2.62	2.50	1.96
CV _t	—	7.44	9.26	8.71	—	9.64

Analyses were performed on a JEOL-733 microprobe using a 10-μm beam and 80-nA beam current (see ref. 16), except analyses on samples from 'E' cores, which were performed on an Etec Autoprobe fitted with an Ortec Si(Li) detector (see ref. 42 for details). Glass standards on both probes included KN-18, UA5831 and VG-99. Total Fe as FeO; water by difference; ND, not detected. Analysis on core E33-16 is representative of the phonolitic shards found in 'ash zone F' in all the 'E' cores examined. Sample numbers and identity are indicated in Fig. 1 and inset. Localities 103 and 208 are the type sections of Mount Curl Tephra¹⁸ and Whakamaru Ignimbrite²² respectively. Other previously named units that were sampled are 165, Rangitaki Ignimbrite⁴⁴; 166, Te Whaiti Ignimbrite⁴⁴; 168, Rangitawa Pumice^{19,43}; and 207, Ohinewai Ash⁴⁵. Ages (in Myr), where determined, are (g) fission track on glass; (k) K/Ar on glass; (z) fission track on zircon; and (s) sedimentation rate based on palaeomagnetic stratigraphy, from the references cited. CV_m and CV_t are the coefficients of variation⁴⁶ for the 8 major and 12 trace elements (La, Ce, Sm, Eu, Tb, Yb, Lu, Th, U, Hf, Sc, Cs) respectively, determined for each sample by comparison with Mount Curl Tephra (site 103).

We present data for the correlation, dispersal and age of a rhyolitic tephra from Taupo volcano, New Zealand. This deposit is larger than any previously reported from Taupo⁹, and appears to represent the largest late Quaternary eruption in the Southern Hemisphere yet documented. As such, the results of this study are relevant to the current interest in atmospheric and climatic responses to the injection of large quantities of fine particles as discussed in recent 'nuclear winter' scenarios¹⁰. Detailed analysis of such responses is beyond the scope of this report.

The Plio-Pleistocene tephrostratigraphy of the south-west Pacific is broadly known¹¹⁻¹⁴, with New Zealand as the assumed source of the predominantly rhyolitic tephra^{11,15}. A succession

of tephra layers, originally labelled A to E, has been recorded in deep-sea cores such as RC9-113 and RC12-215, located up to 1,100 km east of New Zealand^{11,14}. Suggested correlations with terrestrial deposits have been based mostly on palaeomagnetic and fission track dates^{11,14,15}, but glass chemistry has been used recently^{16,17}.

We have determined, by means of electron microprobe¹⁶, the major elements in glass shards from tephra from seven cores taken in the New Zealand region and four cores from the sub-Antarctic (Fig. 1). Each of the seven Pacific Ocean cores has only one tephra whose chemistry matches tephra 'D' from RC9-113. Furthermore, all these 'D'-type tephra are in the same

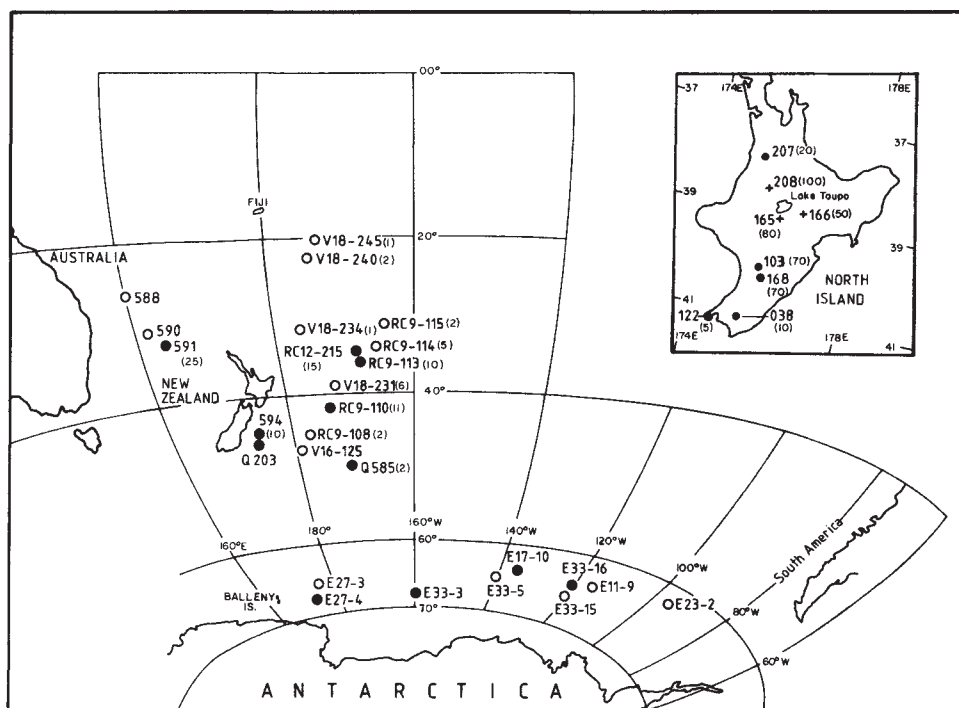


Fig. 1 Location and identity of cores considered in this study to contain Mount Curl Tephra, together with thickness (in cm) where macroscopic. Data from DSDP sites 588–594 from refs 25 and 47. Solid symbols denote cores with an analysed sample (Table 1). Localities on inset map are: ●, airfall Mount Curl Tephra; and +, airfall ash overlain by Whakamaru Ignimbrite.

palaeomagnetic age range of ~230–380 kyr (Table 1). Tephra stratigraphically above or below 'D' (rather than those with a younger or older determined age, but uncertain stratigraphy), have a different glass chemistry to the extent that 'D' is characterized uniquely (Table 1, Fig. 2). The sub-Antarctic cores also contain glass shards and part of 'ash zone F'¹³ has been correlated recently with Whakamaru Ignimbrite on chronological grounds. Interestingly, most of the glass shards from 'F1', 'F2' and 'F3' in cores that we have analysed (E17-10, E27-4, E33-3 and E33-16) are phonolitic (see Table 1 for representative analyses) and did not originate in New Zealand. Less than 20% of all the shards examined are rhyolitic, but these are very fine-grained and, being hydrated, were difficult to analyse accurately. Analyses (Table 1) are similar to 'D', especially with $K_2O > Na_2O$, and are unlike any other tephra of this age¹⁶. We conclude that these shards are tephra 'D' but have undergone some alteration due to the high degree of hydration.

Tephra 'D' has the same major element chemistry (see Table 1; Fig. 2) as the terrestrial Mount Curl Tephra¹⁸—a widespread rhyolitic airfall tephra from Taupo volcano, New Zealand, and also dated at ~230–380 kyr (refs 18–21) (Table 1). As only one tephra with this chemistry and general age has been located in all the terrestrial exposures and marine cores examined, we conclude that tephra 'D' is Mount Curl Tephra, from Taupo volcano. On the basis of further chemical data, Mount Curl Tephra is here regarded as the correlative of the extensive Whakamaru Ignimbrite, a complex sheet of welded ignimbrite in central New Zealand²². Further evidence for this correlation is provided by the rare-earth element patterns in glass separates from samples of the tephra, ignimbrite and tephra 'D' (Fig. 3), which are distinct from those of other, similarly aged tephra.

On land, Mount Curl Tephra is an airfall ash, typified by an abundance of coarse feldspar phenocrysts that are conspicuous in the field, together with hypersthene, hornblende, Fe–Ti oxides and biotite. This same appearance and mineralogy characterizes the most basal (unwelded) portion of Whakamaru Ignimbrite, implying that the crystal-rich layer under the ignimbrite correlates with the airfall tephra. Mount Curl Tephra itself grades up to a fine vitric ash that may be the correlative of the ignimbrite (co-ignimbrite airfall ash).

Detailed carbonate and oxygen-isotope analyses are available for two adjacent tephra-bearing cores, DSDP 594 and Q203

(refs 23, 24), both taken immediately east of New Zealand. Based on the location of the Brunhes/Matuyama palaeomagnetic boundary (730 kyr) at ~99.4 m sub-bottom in DSDP 594 (ref. 25), preliminary results of spectral analyses of the carbonate and isotope records for the entire late Quaternary interval in this core²⁶ reveal the dominant orbital frequencies of about 100, 41 and 23 kyr^{27–29}. Tentative ages can be assigned to all parts of the core, as well as identification of oxygen-isotope stages³⁰ (Fig. 4). In particular, Mount Curl Tephra, at 36.5 m, has a spectral age of 254 ± 2 kyr and lies near the top of glacial Stage 8. This is consistent with the stratigraphic position of the tephra in loess¹⁸ (site 103) and in lignite-containing cool-climate floras³¹ (site 122).

In core Q203, close to New Zealand, the tephra identified as Mount Curl Tephra is just 3.08 m sub-bottom, compared with the 36.5 m for DSDP 594. This difference reflects a major thinning of the sediment cover as evident in seismic profiles (for example, Eltanin 43)³¹. Yet despite this thinning, the glacial

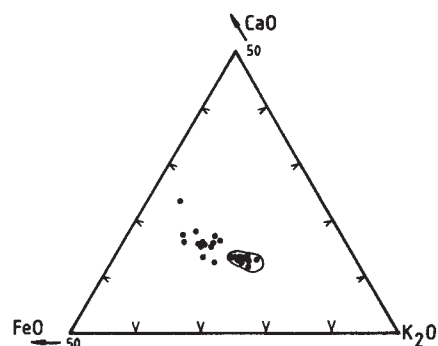


Fig. 2 Proportions of CaO, FeO (total) and K_2O in glass shards from analysed tephra. The encircled samples are Mount Curl Tephra and correlatives, including Whakamaru Ignimbrite and tephra 'D'. The other analyses are from tephra stratigraphically distinct from Mount Curl Tephra, but little different in age, sampled from deep-sea cores and land exposures. The coefficients of variation (CV_m and CV_v) for these other samples, when compared with Mount Curl Tephra (sample 103), range from 10.5 to 25.8 and from 17.4 to 23.8 respectively (compare with values in Table 1).

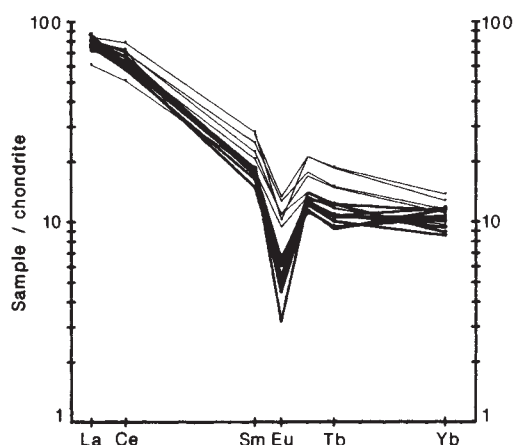


Fig. 3 Chondrite-normalized rare-earth element patterns, determined by neutron-activation analysis⁴⁸, for glass separates from Mount Curl Tephra and correlatives (heavy lines) and for those stratigraphically separate tephras of similar age (light lines) that are also plotted in Fig. 2. Mount Curl Tephra is characterized by a marked negative Eu anomaly and relative depletion of mid and heavy rare-earth elements, probably due to feldspar and hornblende fractionation respectively.

stratigraphy, as evidenced by lithological, microfaunal³³ and oxygen-isotope analysis (Fig. 4) is complete and fully supports the assignment of Mount Curl Tephra to Stage 8.

The location of cores and some terrestrial exposures of Mount Curl Tephra and Whakamaru Ignimbrite (Fig. 1) indicate an extremely widespread distribution, presently limited by the availability of suitable deep-sea cores, especially west and north-east of New Zealand. In addition to the cores that we have sampled, other cores from the Pacific contain ash layers with chronostratigraphic affinity to Mount Curl Tephra. Based on the available information, cores V18-240 and V18-245 contain Mount Curl Tephra¹⁴. From the distribution shown in Fig. 1, we conclude that Mount Curl Tephra was deposited and preserved over at least 10^7 km²—a much greater area than that covered by other documented tephras, such as the 5×10^6 km² covered by the exceptional Toba eruption⁷.

No attempt has been made to draw contours of equal tephra thickness (isopachs), as most of the thickness data are from isolated cores, and in these, tephra thickness is unreliable due to post-depositional processes such as bioturbation, current winnowing and gravitational mass movement³⁴. A broad estimate of volume yields a conservative 700 km³. The volume of Whakamaru Ignimbrite is similarly difficult to estimate, but is at least 500 km³ (ref. 22) and probably "of the order of $>1,000$ km³" (see ref. 35). The minimum total volume of tephra is 1,200 km³ but probably nearer 2,000 km³, or at least 1,500 km³ of magma, making it, without doubt, the largest documented eruption from the Southern Hemisphere and one of the largest late Quaternary eruptions known. This eruption from Taupo, New Zealand, eclipses the extensively documented 1.8-kyr eruption of Taupo³⁶⁻³⁸.

The occurrence of tephra in the deep-sea cores, both east and south of New Zealand, confirms that volcanic ash transport was dominated by westerly winds and poleward atmospheric circulation. It is doubtful that surface sea currents, dominated by the West Wind Drift, could distribute such a large volume of tephra, composed of dense platy shards, rapidly enough to form pure, undiluted macroscopic layers or concentrations of shards. Distribution to the north and west is counter to both the poleward circulation and the prevailing westerly winds. However, direct northwestward transport is possible by stratospheric southeasterly winds which today prevail above 20-km altitude between September and March (J. W. D. Hessel, personal

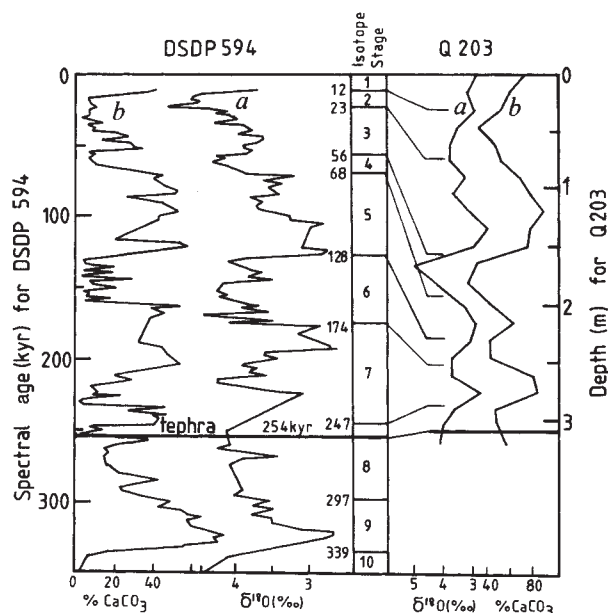


Fig. 4 A portion of the benthic foraminiferal $\delta^{18}\text{O}$ (a) and CaCO_3 (b) records²⁴ for the late Quaternary sequence plotted as a function of age derived from spectral analyses for DSDP 594 and as a function of depth for core Q203. The timescale is based on the location of the Brunhes/Matuyama boundary (730 kyr) at 99.4 m sub-bottom in core 594 (ref. 25) and has been tuned to the orbital perturbation spectra using the forcing frequencies 100, 41 and 23 kyr²⁶. The resulting dates of isotope stage boundaries 1/2 to 9/10 match closely those determined recently³⁰. The scheme dates Mount Curl Tephra at ~ 254 kyr.

communication), contemporaneous with lower-level westerly winds. The observed dispersal implies that ash reached the stratosphere.

Injection of large quantities of material into the stratosphere, to heights above 20 km, must have had a major impact on the atmosphere and possibly the climate of the South Pacific. In the absence of other data, it is tempting to ascribe the short cooling event in the oxygen-isotope curve immediately above the tephra to this cause. Recent work suggests that the quantity of sulphate aerosols is crucial to determining the climatic effect of volcanic eruptions^{1,39,40}, although observed temperature decreases are greatest within 1–2 months of an eruption, while the atmosphere is still laden with fine ash⁴¹. The quantity of fine ash and sulphur injected into the stratosphere by this large eruption of Taupo is unknown, but an estimate can be made using recent analogies⁴⁰. If as little as 0.1% formed $<2\text{-}\mu\text{m}$ particles¹, 2×10^{10} tonnes would have been generated, compared with 1.6×10^8 tonnes for Krakatau and 1.2×10^9 tonnes for Tambora¹. This represents more dust than is postulated by nuclear scenarios¹⁰.

Given the widespread distribution of tephra (Fig. 1) and the rapidity of atmospheric transport⁴, it is likely that material from this eruption of Taupo was deposited around the globe; a contention that should be tested by investigation off South America, 10,000 km from the volcano.

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Aeromagnetic anomalies and tectonic trends in and around the Benue Trough, Nigeria

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Four major fracture zones cutting the Atlantic sea floor off the west coast of Africa—St Paul's, Romanche, Chain and Charcot—have been delineated offshore using bathymetric, seismic, gravity and magnetic data, respectively¹. But the extrapolation of these fractures onto the African Continent is uncertain. Here we analyse recently compiled aeromagnetic data² for the Benue Trough and adjacent areas of Nigeria, and demonstrate the existence of narrow magnetic lineaments parallel to the margins of the Benue Trough, which can be matched with the oceanic fractures. Alkaline ring complexes which predate the opening of the Atlantic Ocean are concentrated along these lineaments, suggesting that they represent pre-existing zones of weakness in the African crust which may have influenced the rifting of the continental margins and the early development of Atlantic fracture zones.

The aeromagnetic data extend from 7 to 12° E longitude and from 7 to 12° N latitude (Fig. 1), and were compiled from aeromagnetic maps recently released by the Geological Survey of Nigeria². A contoured map (Fig. 2) of the total magnetic field residuals has magnetic values ranging from 31,995 to 33,250 nT and an average value of 32,860 nT. To investigate the significance of these data, they were filtered and continued upwards. One of the most striking features is a series of long sub-parallel magnetic anomaly belts trending ENE–WSW, designated as AA' to FF' (Fig. 2). The magnetic lineaments show up as continuous narrow belts (10–15 km in width) of negative anomalies ranging in length from 10 to 650 km and in amplitude from 150 to 250 nT. They are of sufficient prominence and length to suggest that they are probably the signatures of regional structural features representing major tectonic trends, because they cut across both basement and sedimentary rocks in the study area. MAGSAT data across West Africa also show anomaly trends over the sea floor overlapping the continent³. These aeromagnetic results should, therefore, be considered in the context of a continent-wide tectonic setting.

The Benue Trough, which lies in the centre of this area (Fig. 1), is considered a rift valley, and is filled with >5 km of Tertiary and Cretaceous sediments. It has very large width (120–200 km) when compared with widths (59±15 km) normally associated with rift valleys⁴. The trough is believed to have originated as a tensional graben system during the Cretaceous, and to have experienced a period of compressional deformation at the end of the Cretaceous resulting in the folding of the sediments parallel to the axis of the trough^{5–7}. It connects with a pattern of Cretaceous troughs to the north of Niger (east of Air) and to the east across Chad to Sudan⁸.

The pattern of the magnetic lineaments follows the linear margins expected for a rift. The lineaments CC' and DD' (Fig. 2) mark the structural boundary of the trough. They also match the onshore extensions, under the Niger Delta, of the Chain and Charcot oceanic fractures respectively (rather than the Charcot fracture alone as has been suggested^{9,10}). This inference supports an earlier suggestion¹⁰ that the Benue Trough may have been initiated by the extension of the equatorial fracture zones onto the continent during the early stages of the separation of Africa and South America. Also there is agreement with Benkhellil¹⁰ that there exists a narrow fault zone within the basin, which is indicated on the magnetic anomaly map (cc' in Fig. 2).

With the boundary of the trough defined by lineaments CC' and DD' (Fig. 2), the structure of the north-eastern section of the study area appears more complicated. For example, the Tertiary Kerikeri formation lies mostly outside these boundaries (Fig. 1). The magnetic data suggest a faulted contact between the north-eastern Cretaceous sediments in the trough as we define it and the south-eastern boundary of the Kerikeri formation.

The Nigerian Basement Complex, in which the Benue Trough lies, consists of Precambrian rocks that were faulted and fractured before and during the Pan-African orogeny with many of the granites yielding this latter age. The most prominent fracture direction is N–S (products of brittle deformation) on which is superposed a conjugate system of strike-slip NE–SW and NW–SE faults believed to have resulted from transcurrent movements¹¹. The Basement Complex in the north-west part of the study area is an uplifted zone intruded by alkaline ring complexes with an age range 141–213 Myr (ref. 12).

The magmatism responsible for these ring complexes has been related to the action of mantle plumes beneath the lithosphere^{13–15} or to intraplate deformation with reactivation of deep fractures^{9,16–18}. It has been suggested¹⁹ that there is an intense correlation between periods of rapid plate motion and intra-

Fig. 1 Geological map of Nigeria, showing the study area, the locations of the inferred lineaments and faults, and the geological units. Younger Granite complexes: 1, Jos-Bukuru and Jarawa; 2, Amo and Buji; 3, Afu; 4, Ningishi; 5, Sha-Kaleri; 6, Pankshin; 7, Langtang. XY, Location of the profile shown in Fig. 3.
 ▨, Quaternary sandstone;
 ▩, tertiary sandstone;
 ▧, cretaceous sandstone;
 ■, younger granite series;
 ▤, Precambrian-Cambrian rocks;
 ---, inferred lineaments/faults.

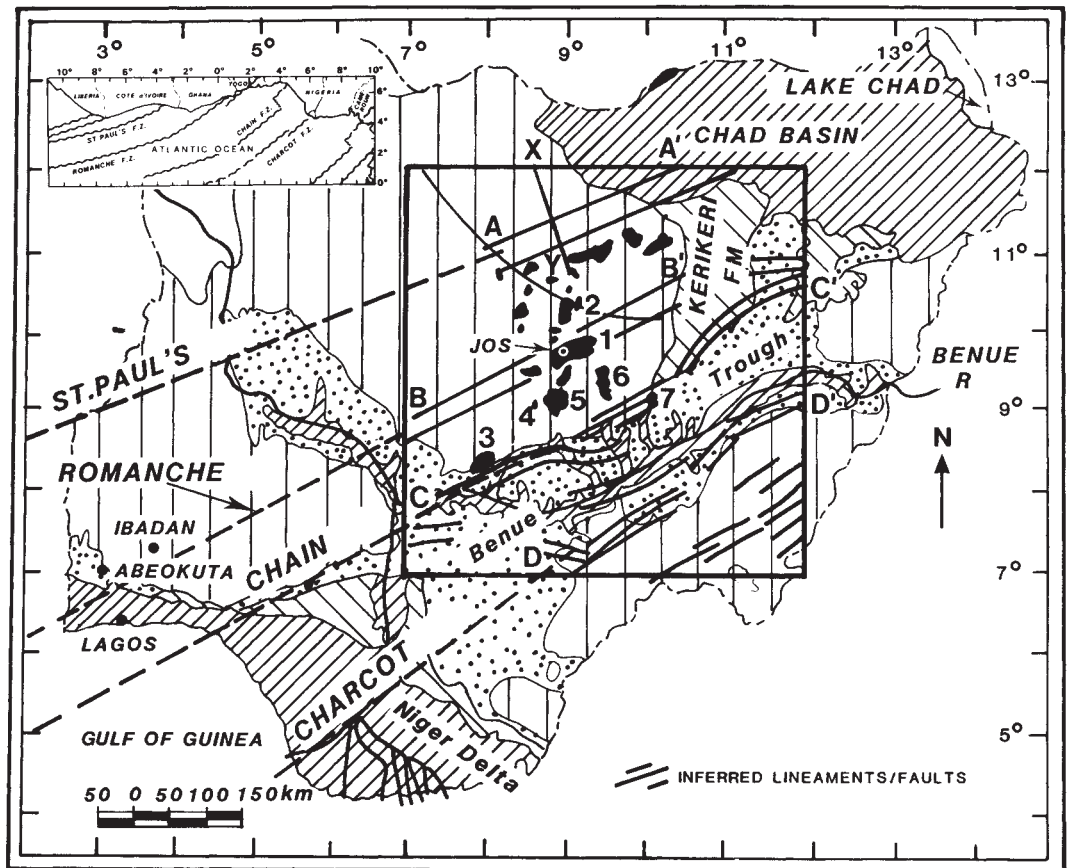


plate magmatic activity which may correspond to interference and collision with the African plate¹⁸.

Alkaline ring complexes have also been interpreted as being related to rifting^{9,18,19} and to transform faulting^{9,20}. Mainly because alkaline ring complexes have been found to align along transform directions^{9,18}, they are thought either to lie along fractures which precede and predetermine the location of oceanic transform faults, or to have occurred later during reactivation of the fractures situated at the ends of oceanic transform faults. In the case of the Nigerian ring complexes, which are a few million years older than the onset of seafloor spreading in the South Atlantic, it has been proposed⁹ that the ring complexes reflect an early stage of rifting between Africa and South America that preceded the development of the first oceanic crust by a few tens of millions of years.

The magnetic data support the correlation of this intra-plate magmatism with transform faulting. In the north-eastern section of the Basement Complex of the study area (Figs 1 and 2) a major feature is depicted by a NE-SW linear zone of positive magnetic anomalies (AA', Fig. 2) flanked on either side by negative anomalies. This zone runs along the north-western boundary of the Kerikeri formation at its contact with the Chad formation and across the central crystalline shield area, where it is weakly developed. If this zone is linked to the St Paul's fracture zone, the contact must be faulted. Preliminary interpretation²¹ of anomalies (Fig. 3) along this trend agrees quantitatively with this model.

The second lineament in this region (BB', Fig. 2) is aligned along a continental extension of the Romanche fracture zone in the mid-Atlantic and cuts across the Tertiary Kerikeri formation and the Cretaceous sediments. The lineament has very little surface expression. The prominent anomaly in the centre of BB' (Fig. 2) and other short-wavelength anomalies (10–30 km) over the crystalline shield and south of the line bb' have been shown to reflect surface or near-surface younger granite intrusives^{2,22}.

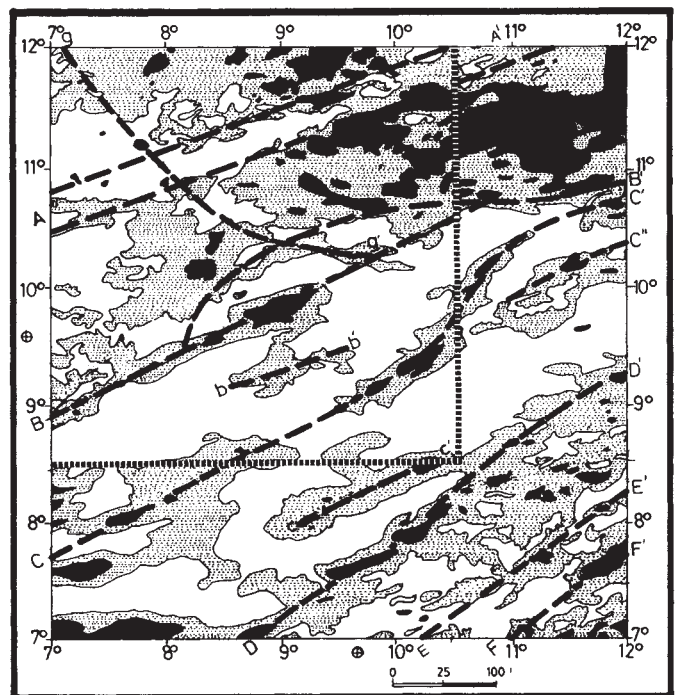


Fig. 2 Total-field aeromagnetic map of the study area, showing the lineament trends (field values are relative to a 25,000 nT base). DD', CC', BB', AA' are correlated with the Charcot, Chain, Romanche and St Paul's fracture zones in the mid-Atlantic (see also Fig. 1). All important trends are given letter designations, although not all of these are referred to in the text. The area outlined in the north-western portion of the map has been previously published². The intensity ranges in nT are: □, $\geq 7,860$; ▨, 7,780–7,860; ■, $\leq 7,780$. ▤, Inferred lineaments/faults.

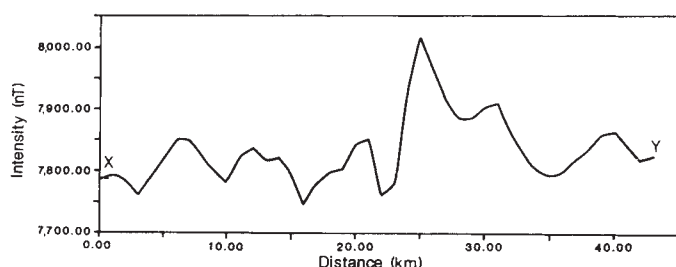


Fig. 3 Total field profile along XY (Fig. 1). A faulted contact is indicated at that location, consistent with the inferred continental lineament or fault associated with the St Paul's fracture zone.

The anorogenic alkaline ring complexes appear to be concentrated on the WSW-ENE trending magnetic lineaments. The connection between fracture zones and complexes suggests that these palaeo-megacontinental faults existed before the emplacement of the complexes and that they were reactivated in that period, either due to pressure from an underlying magma chamber or through tensional stresses in the lithosphere that allowed the release of magma to the surface. Thus, it can be inferred that the Romanche fracture zone extension along lineament BB' was reactivated on the Nigerian continental mass just before 161 Myr, which is about the age¹² of the Kagoro, Jos-Bukuru and Jarawa complexes lying along it (Fig. 1). Ages of ~151 Myr (ref. 12) for the aligned Ningishi, Sha-kaleri and Pankshin complexes may represent another such rejuvenated period (lineament bb', Fig. 2), while the period of activity around 141 Myr (ref. 12) of the Afu and Langtang complexes (lineament CC', Fig. 2) could mark a period of reactivation of the continental fracture zone associated with the Chain fracture system. This supports Sykes¹⁹ suggestion that various types of pre-existing zones of weakness, such as fault zones, suture zones, continental rifts, aulacogens and monoclines, have pronounced influence on the shape of subsequent continental margins and the locations of oceanic transform faults.

A further study of the magnetic anomalies centred on the complexes² and previous interpretations of gravity data¹⁹ show a subsurface outline of the complexes which is commensurate with the idea that these diapirs may have risen from a common deep-seated source, possibly located in the mantle.

The existence of fracture zones extending from the continent to oceanic transform faults is also corroborated by other observations. First, the few recorded earthquakes in this area (in 1906 and 1939 in Accra, Ghana²³, and three earthquakes in 1984 near Ibadan, Nigeria) lie on faults which seem to extend from the continent to the Romanche Fault²⁴. Second, this area shows up on LANDSAT imagery as a continent-wide shear system (Pelusium line) with its associated lineament system^{25,26}. Third, these lineaments parallel structural trends in the oceanic crust off West Africa^{2,27} and other parts of West Africa²⁸ and Eastern Brazil²⁹.

In conclusion, the Nigerian continental mass contains lineaments with definite magnetic signatures which are enhanced by the presence of anorogenic ring complexes. Further, these lineaments have a one-to-one relationship with the St Paul, Romanche, Chain and Charcot fracture zones in the continental margins of West Africa. Thus the oceanic fracture zones do not curve to the north-east on approaching the north coast of Guinea and then terminate at a relatively short distance inland as has been previously suggested^{27,30}. Our evidence supports the hypothesis that major continental fracture zones tend to develop near pre-existing zones of weakness inherited from the last orogeny⁹ in the continent. Thus, the magnetic lineaments could be considered to be part of the signatures of megalineament-geosutures, shear zones^{20,31} or zones of weakness, that predate³² the opening of the Atlantic Ocean. These zones were reactivated during the early stages of continental rifting, parts of which became foci of ocean spreading³².

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High-pressure transformation of eclogite to garnetite in subducted oceanic crust

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The mineral composition of oceanic crust is a function of pressure and temperature and thus changes as a slab of crust descends a subduction zone. A knowledge of the mineralogy of subducted material would provide important constraints on its geophysical characteristics. We have sought, by means of high pressure laboratory experiments (in the pressure range 4.6-18 GPa) on a synthetic mix with the composition of mid-ocean ridge basalt (MORB), to determine the sequence of mineralogical changes. We find that at 1,200 °C, solid solution of pyroxene in garnet begins around 10 GPa, and increases substantially between 12 and 15 GPa. Complete transformation of eclogite to garnetite is achieved at 15 GPa. The garnetite has a zero-pressure density of 3.76 g cm⁻³ and accordingly would be denser than the surrounding mantle throughout its stability field, which extends to depths of ~600 km. If subducted to greater depths, garnetite may transform to perovskite.

A model basaltic starting composition was prepared from a mixture of appropriate proportions of oxides, hydrates, carbonates and metal, and melted completely above its liquidus under an argon atmosphere before quenching in cold water to yield a glass starting material. Its composition, in weight per cent, is

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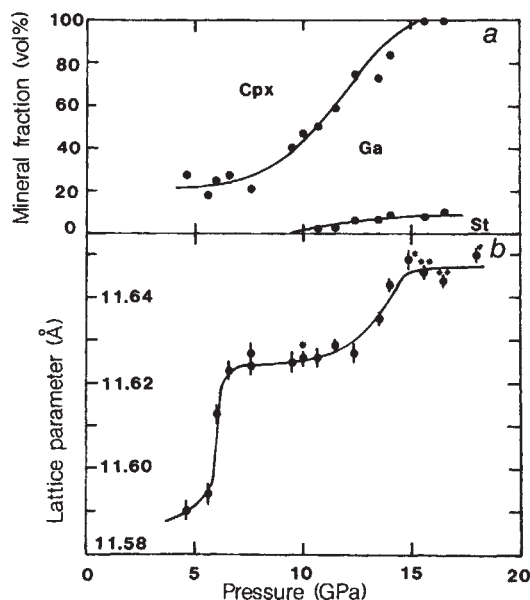


Fig. 1 *a*, Mineral proportions in a typical primitive MORB composition vary systematically with increasing pressure. *b*, Garnet lattice parameters increase non-linearly with increasing pressure. MORB composition starting materials are glass, pyroxenite (*) or amphibolite (**). Vertical bars indicate estimated uncertainties.

50.4 SiO₂, 0.6 TiO₂, 16.1 Al₂O₃, 7.7 FeO, 10.5 MgO, 13.1 CaO, 1.9 Na₂O and is equivalent to that of a typical primitive MORB¹. The homogeneity of the glass was confirmed optically and by electron-probe microanalysis. Pyroxenite and amphibolite crystallized from the glass at 1.0–1.5 GPa, 900 °C, were used as starting materials in some runs to minimize metastability problems (see Fig. 1).

High-pressure experiments were carried out using a pair of Bridgman anvils from 4.6 to 12 GPa and an MA-8 system at 10 GPa and above. Detailed experimental methods and procedures will be described elsewhere^{2,3}. Pressure was calibrated at 1,200 °C using several well-determined phase boundaries, namely Fe₂SiO₄ olivine-spinel, coesite-stishovite, and Mg₂SiO₄ olivine-modified spinel. Uncertainty in the pressure measurement is believed to be ±0.5 GPa. Temperature was measured using Pt/Pt-10%Rh thermocouples without correcting for the effect of pressure on e.m.f. All runs were carried out at 1,200 °C except one at 6.6 GPa and 1,100 °C. Runs lasted from 30 to 90 min. Quenching was achieved by cutting off the heating power input. The products were examined optically, by X-ray diffraction and by electron microprobe. Lattice parameters of garnets were determined from the (14, 4, 0) and (14, 4, 2) reflections. The proportions of minerals in the run products were estimated from X-ray diffraction line intensities and by mass balance using electron microprobe analyses of individual phases.

Figure 1*a* summarizes how mineralogy changes as a function of pressure. Above 15 GPa the product assemblage consists of single-phase garnet, while stishovite was identified in runs between 12 and 18 GPa. Figure 1*b* illustrates how garnet lattice parameters vary with pressure. They increase sharply around 6 GPa, then pass through a plateau between about 8 and 12 GPa, followed by another increase between 12.5 and 15 GPa, to level out between 15 and 18 GPa at ~11.65 Å.

The CaO content of garnet changes in a manner analogous to the lattice parameter variations, with a rapid increase at around 6 GPa by 6 mol %, followed by a more gentle increase between 12 and 15 GPa. Solid solution of pyroxene components in garnet begins around 10 GPa and increases substantially between 12 and 15 GPa. Garnet contains <0.7 wt% Na₂O below 10 GPa, and 1.0–1.6 wt% at higher pressures. Clinopyroxenes

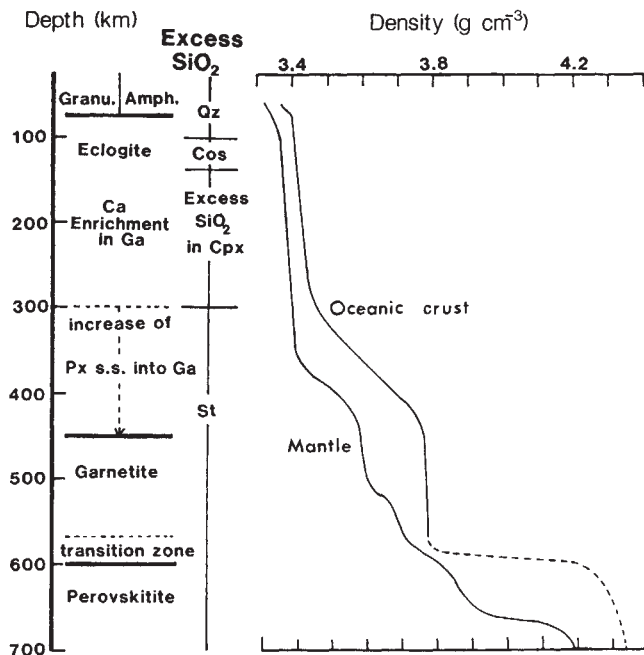
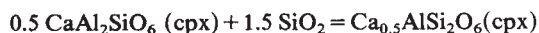


Fig. 2 Subsolidus phase changes and calculated zero-pressure densities of subducted oceanic crust are shown as a function of depth. The presence or absence of a free SiO₂ phase in the assemblage is also indicated. For comparison, the density of surrounding mantle (pyrolite composition) is shown⁶. Granu, granulite; Amph, amphibolite; Ga, garnet; Px s.s., pyroxene solid solution; Qz, quartz; Cos, coesite; Cpx, clinopyroxene; St, stishovite.

become more jadeitic, and less aluminous with increasing pressure. The 'excess SiO₂' content of clinopyroxene, calculated as a Ca_{0.5}AlSi₂O₆ molecule, remains essentially constant at 16 ± 2 mol % up to 9.5 GPa, but decreases sharply thereafter. This pressure corresponds to the coesite-stishovite transition. Calculated molar volume changes (ΔV s) for the reaction



confirm that 'excess SiO₂' is accommodated in solid solution in clinopyroxene in the coesite stability field ($\Delta V = -2.5 \text{ cm}^3 \text{ mol}^{-1}$), but exsolves as free stishovite within the stishovite stability field ($\Delta V = +7.6 \text{ cm}^3 \text{ mol}^{-1}$).

Ringwood⁴ reported lattice parameter and modal abundance variations for garnets crystallized from basaltic glasses at pressures up to 17 GPa. His data indicate a discontinuity in lattice parameter at around 5 GPa, corresponding to the sharp increase in lattice parameters and CaO content reported in this study. His experiments on an alkali-poor olivine tholeiite yielded a garnetite (>95% garnet) coexisting with a small amount of jadeitic clinopyroxene at ~13 GPa, 900 °C. Liu⁵ reported lattice parameter variations for garnets and proportions of phases crystallized from a basaltic composition very similar to that used in this study. Allowing for the large uncertainties involved in diamond-anvil pressure calibration, his data and ours are also found to be consistent. Liu reported that the ratio of garnet to clinopyroxene progressively increased with increasing pressure between 10 and 15 GPa, while X-ray diffraction studies of an 18 GPa product assemblage revealed only a weak garnet spatter, with no clinopyroxene lines. X-ray diffraction patterns of quenched 21- and 28-GPa runs contained only a few faint lines, from which he inferred that the high-pressure phase assemblage in this interval consisted of an unquenchable perovskite-type phase.

Figure 2 illustrates the subsolidus phase changes and corresponding density increases in subducted oceanic crust to a depth

of 700 km, using data from this study and from Liu⁵. The MORB basaltic composition undergoes three major phase transformations: to eclogite at around 80 km, from eclogite to garnetite at around 450 km, and from garnetite to perovskite at around 600 km. Substantial solid solution of pyroxene components in garnet begins at a depth of 300 km and is essentially completed by 450 km. The behaviour of 'excess SiO₂' is also indicated in Fig. 2. At depths shallower than 150 km, the eclogite contains free quartz or coesite. However, between 150 and 300 km, 'excess SiO₂' is accommodated in solid solution in pyroxene as a Ca_{0.5}AlSi₂O₆ component so that there is no free SiO₂ phase. At depths below the coesite-stishovite transition, stishovite appears in the phase assemblage.

Figure 2 also shows how the calculated density of subducted basaltic crust increases with depth. The calculations use mineral compositions as determined from electron-probe microanalysis and garnet lattice parameters (Fig. 1). The proportions of garnet and clinopyroxene were deduced from mass-balance considerations. Clinopyroxene densities were calculated using compositions determined by electron-probe microanalysis and assuming ideal solid solution between diopside, clinoenstatite, clinoferrrosilite, jadeite, Ca-Tschermak and Ca_{0.5}AlSi₂O₆ endmembers. The individual zero-pressure densities of both garnet and clinopyroxene decrease almost linearly with increasing depth. The increase in density of the oceanic crust is caused by increasing the proportion of garnet to clinopyroxene, as shown in Fig. 1. Note that the abrupt increase in the CaO content of clinopyroxene at 6 GPa (200 km) does not result in any appreciable increase in density. Figure 2 shows that only a modest increase in density is experienced by subducted basaltic crust

down to depths of ~300 km. However, between about 350 and 400 km there is a rapid increase, reaching 3.76 g cm⁻³ by 450 km where complete transformation to garnetite is attained. The density change throughout the garnetite stability field is negligible; however, the transformation of garnetite to perovskite is probably accompanied by a substantial and rapid increase in density, as indicated by the dashed portion of the curve.

Our results demonstrate that subducted oceanic crust would remain denser than surrounding mantle (assumed to possess a pyrolite or peridotite composition) between depths of 60 and 600 km. Moreover, the results of Liu⁵ imply that this situation prevails in the 600–700 km depth interval, so that gravitational body forces should cause former oceanic crust to penetrate the 670-km seismic discontinuity. According to Ringwood⁶, however, harzburgite, which comprises the main part of the oceanic plate and underlies the oceanic crust, may also be denser than surrounding mantle down to around 670 km depth, but becomes less dense below this depth. Thus, the main part of a subducted lithospheric plate probably stops its further penetration at around this depth. These relationships have important geodynamic implications^{6,7}.

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The pyroxene paradox in MORB glasses—a signature of picritic parental magmas?

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A persistent conclusion reached in studies of glass in the chilled margins of mid-ocean ridge basalts (MORB) is that occult clinopyroxene fractionation is required in order to explain the observed variation in glass compositions¹. Successful crystal-fractionation models commonly require crystallizing assemblages which are dominated by plagioclase and clinopyroxene rather than the plagioclase and olivine which dominate the phenocryst assemblages. The 'pyroxene paradox' arises from the observation that clinopyroxene phenocrysts are uncommon in lavas containing more than 8.5 wt% MgO (refs 2, 3) and the fact that melting experiments on liquids with such compositions indicate that clinopyroxene appears only at 20–40 °C below liquidus temperatures^{4–7}. Based on a study⁸ of compositional relationships between glasses and chilled margins of pillow lavas from the Baffin Bay picrite suite, I suggest here that the 'pyroxene paradox' is an illusion which arises in part because of the compositional dispersion of olivine fractionation trends from picritic parental magmas between the limiting cases of equilibrium and fractional crystallization.

The Baffin Bay volcanic province consists of a succession of Palaeocene⁹ basaltic lavas that erupted during the opening of the Davis Strait separating Greenland from Baffin Island. The lavas occur as a series of outcrops between Cape Searle and Cape Dyer along the eastern coast of Baffin Island and between the Svartenhuk Peninsula and Disko Island along the western coast of Greenland. The lower part of the succession at Padloping Island⁸ contains abundant pillow lavas and pillow breccias

which retain fresh volcanic glass in their chilled margins. All these glasses contain millimetre-sized olivine phenocrysts, with inclusions of euhedral spinel, and plagioclase microlites but no clinopyroxene. Although there is a systematic increase in the bulk MgO content of the chilled margins with the abundance of olivine phenocrysts, there is also a general increase (Fig. 1) in the forsterite content of the olivine phenocrysts with the MgO content of both coexisting glasses and corresponding chilled margins. This correlation rules out a mechanism for formation of the more magnesian lavas involving the simple mechanical accumulation of the olivine phenocrysts observed in liquids of lowest MgO content.

I have shown⁸ that despite the complications of olivine accumulation, the presence of extremely forsteritic olivine megacrysts (Fo 93+) argues for the existence of liquids whose compositions are approximated by the bulk compositions of the more magnesian lavas such as sample Pd-19 (Table 1). Fractional crystallization of olivine from a magma of the same composition as Pd-19 could produce a succession of liquid compositions which approximate the compositional spectrum of the lava chilled margins (Fig. 2, trend *a*). For example, the composition of the chilled margin of Pd-14 can be obtained by extracting 30% olivine with minor spinel from the composition of Pd-19 [Table 2, model (1)]. A strong component of equilibrium crystallization (Fig. 2, trend *b*), however, is required to explain the composition of the glass actually found in the chilled margin of Pd-19. In reality, a magma of Pd-19's composition may have evolved along any path between the limiting cases of perfect fractional crystallization (trend *a*) and perfect equilibrium crystallization (trend *b*), depending on the local dynamics of the magma-chamber processes which controlled crystal/liquid separation.

The interesting question is what controls the spectrum of glass compositions. The presence of plagioclase microlites in the glasses of all the pillow lavas, regardless of bulk margin MgO content, indicates that the liquids of these lavas lay on the olivine-plagioclase cotectic at the time of their eruption. In studies of MORB, glass trends such as that from Pd-19 to Pd-14

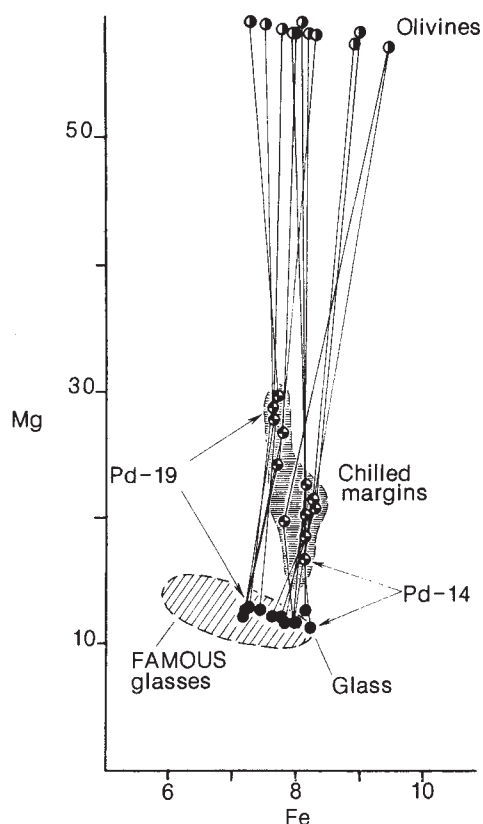


Fig. 1 Mg-Fe plot, in cation units, of the compositions of olivines (●), glass (●) and chilled margins (●) of Padloping Island pillow lavas. The tie-lines join coexisting phases. The horizontally shaded field indicates the compositional range of 48 Padloping Island lava chilled margins (after Francis⁸) and the diagonally shaded field indicates the compositions of glasses from the FAMOUS project.

(Fig. 2, trend *c*) are commonly interpreted to reflect crystal fractionation trends. Model mixing calculations using only olivine and plagioclase [Table 2, model (2)] yield mineral compositions which are clearly unrealistic (that is, plagioclase is required to be pure anorthite and olivine pure forsterite) to produce the glass of Pd-14 from that of Pd-19. Finite-difference computer models¹⁰ using instantaneous equilibrium compositions for plagioclase¹¹ and olivine¹² yield a rise of Ca content with fractionation, a feature which is not observed along the spectrum of glass compositions. Mixing models which include clinopyroxene are thus significantly better [Table 2, model (3)], although they involve a much larger degree of fractionation and a rather Fe-rich clinopyroxene. Such models require the extraction of significantly more pyroxene than olivine in order to prevent a build-up of Ca. Clinopyroxene is, however, absent from the Padloping glasses and we face a pyroxene paradox similar to that commonly reported in studies of MORB glasses.

The paradox arises because of the assumption that the spectrum of glass compositions represents a trend of compositional evolution. The disposition of the tie-lines in Fig. 1 suggests instead that each glass marks the arrival of an individual olivine fractionation path at the plagioclase-saturation surface. In the Padloping lavas, the length of the glass spectrum in Mg-Fe space approximates the separation, at plagioclase saturation, of the two limiting trends for olivine fractionation from an initial magma of Pd-19's composition (Fig. 2A). Although the presence of plagioclase microlites requires that some plagioclase crystallized from these liquids at eruption, there is no need to propose that glasses similar to Pd-14 fractionated from glasses similar to Pd-19 along the plagioclase-saturation surface. In such a model, no clinopyroxene is required to explain the lack of Ca

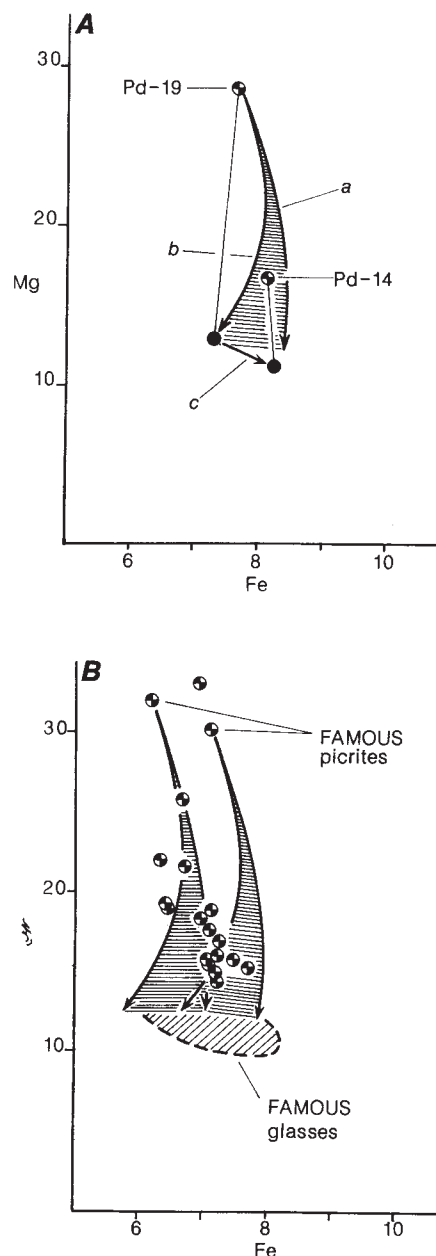


Fig. 2 A, Mg-Fe plot, in cation units, indicating calculated crystal fractionation paths. Trend *a* represents the path of Pd-19 undergoing perfect fractional crystallization of olivine. Trend *b* represents a perfect equilibrium olivine crystallization path for Pd-19. The low-Mg ends of paths *a* and *b* terminated at the point of plagioclase saturation, calculated using the technique of Neilson and Dungan¹¹ with olivine calculated after Ford *et al.*¹², and $\text{Fe}^{3+}/\Sigma \text{Fe}$ fixed at 0.10. Trend *c* represents the path of crystallization of olivine, plagioclase and clinopyroxene from glass Pd-19 to glass Pd-14 as in model (3) of Table 2. B, Mg-Fe plot, in cation units, showing the compositions of MORB picritic lavas from FAMOUS^{20,22} and DSDP³ sites. The heavy arrows indicate the fractional and equilibrium olivine crystallization paths (calculated as in A) for two FAMOUS picrites to the point of plagioclase saturation. Note the correspondence between the plagioclase-saturation points at the ends of the fractionation arrows and the diagonally shaded field of FAMOUS glass compositions.

variation across the spectrum of glass compositions. Calcium is essentially an incompatible element during olivine extraction and its behaviour is unaffected by the mode of fractionation. Thus, with the recognition that the Padloping magmas fractionated to the spectrum of glass compositions by the extraction of

Table 1 Chemical compositions of magnesian lavas

	Pd-14			Pd-19		
	Margin	Glass	Olivine	Margin	Glass	Olivine
SiO ₂	46.94	50.80	39.59	44.92	49.79	40.49
TiO ₂	1.17	1.46	—	0.79	0.93	—
Al ₂ O ₃	14.22	14.22	—	10.52	15.33	—
Cr ₂ O ₃	0.10	—	—	0.25	0.06	—
MgO	11.97	8.03	45.79	21.94	9.27	48.15
FeO	10.39	10.64	13.57	10.32	9.45	11.01
MnO	0.18	0.19	0.19	0.17	0.16	0.15
NiO	0.03	—	0.26	0.11	0.02	0.37
CaO	11.75	12.75	0.32	9.00	13.01	0.28
Na ₂ O	1.64	2.24	—	1.02	1.88	—
K ₂ O	0.05	0.21	—	0.03	0.06	—
P ₂ O ₅	0.08	0.15	—	0.05	0.17	—
LOI	0.93	—	—	0.86	—	—
Total:	99.45	100.69	99.72	99.98	100.13	100.45
Cation units						
Si	43.76	47.00	33.02	40.33	45.98	33.14
Ti	0.82	1.02	—	0.53	0.65	—
Al	15.62	15.51	—	11.13	16.69	—
Cr	0.07	—	—	0.18	0.04	—
Mg	16.63	11.07	56.92	29.36	12.76	58.73
Fe	8.10	8.23	9.46	7.75	7.30	7.54
Mn	0.14	0.15	0.13	0.13	0.13	0.10
Ni	0.02	—	0.17	0.08	0.01	0.24
Ca	11.74	12.64	0.29	8.66	12.87	0.25
Na	2.96	4.02	—	1.78	3.37	—
K	0.06	0.25	—	0.03	0.07	—
P	0.06	0.12	—	0.04	0.13	—
O	151.01	153.81	133.02	145.67	153.47	133.14
Oliv. Fo	87.5	81.3	85.7	93.2	85.1	88.6
	(calc.)	(calc.)		(calc.)	(calc.)	

Bulk pillow margin X-ray fluorescence analyses were performed by Tariq Ahmedali at McGill University with a Phillips PW 1400 instrument using fused disks and an α coefficient technique. Electron microprobe analyses of glass and olivines were performed on a Cameca CAMBAX/Micro system at McGill University using the MBXCOR/Micro software package. Total Fe was calculated as FeO and the olivine compositions were calculated according to the method of Ford *et al.*¹². Analysis sets for 10 other Baffin pillow lavas are presented elsewhere⁸. LOI means loss on ignition.

Table 2 Fractionation models

Model (1)	Bulk margin Pd-19 to bulk margin Pd-14					
	Pd-19 = Pd-14 + olivine (Fo 89.8) + chromite					
	(100)	(70.0)	(29.4)	(0.6)		
	$\Sigma (\text{residuals})^2 = 0.23$					
Model (2)	Pd-19 glass to Pd-14 glass					
	Pd-19 = Pd-14 + olivine (Fo 100) + plagioclase (An 100)					
	(100)	(87.9)	(4.5)	(7.7)		
	$\Sigma (\text{residuals})^2 = 0.23$					
Model (3)	Pd-19 glass to Pd-14 glass with clinopyroxene					
	Pd-19 = Pd-14 + olivine (Fo 81.3) + plagioclase (An 78.5)					
	(100)	(63.5)	(6.0)	(19.2)		
	+ clinopyroxene (Wo 32.4, En 44.0, Fs 23.6)					
	(11.3)					
	$\Sigma (\text{residuals})^2 = 0.01$					

Values in parentheses are percentages. Calculations (in cation units) were performed with the mineral distribution program of Wright and Doherty²¹. The compositions of plagioclase, olivine and clinopyroxene were calculated using the program from ideal end-members. In model (3) the olivine composition was fixed at that which would equilibrate with the glass of Pd-14.

olivine from picritic parents rather than along the glass spectrum via the fractionation of plagioclase, clinopyroxene and olivine, the pyroxene paradox disappears.

Accepting the above, one wonders whether a similar conceptual problem underlies the pyroxene paradox in MORB. Explanations for the pyroxene paradox have ranged from magma mixing^{13,14} to clinopyroxene fractionation at depth¹⁵. More significantly, the work of Bryan *et al.*^{16,17} indicates that the illusion of the pyroxene paradox may simply reflect the existence of multiple olivine-phyric parental magmas. The point here is that if MORB have picritic parental magmas, much of the compositional variation that is responsible for the pyroxene paradox may be attributed to the dispersion of olivine fractionation trends between the limits of equilibrium and fractional crystallization. Clarke¹⁸ has argued that the picritic lavas of Baffin Bay could represent the parental magmas for MORB. However, as pointed out by Langmuir and Hanson¹⁹ and indicated in Fig. 1, the Baffin picrites are too Fe-rich to be suitable parental magmas for MORB. The picritic lavas from MORB localities are, however, systematically Fe-poor compared with the Baffin picrites⁸. Figure 2B shows the results of assuming that two picritic lavas represent analogues of parental magmas for the glasses sampled at the FAMOUS site. In a dynamic open-system conduit system, primary magmas of compositions between these two picrite compositions could evolve to plagioclase saturation by olivine fractionation along a variety of paths between the limiting cases of equilibrium and fractional crystallization. Although a range of picritic magma compositions seems to have existed in the FAMOUS area, much of the elongation of the spectrum of FAMOUS glasses in Mg-Fe space could simply reflect this dispersion effect rather than crystal fractionation along a plagioclase-clinopyroxene-olivine cotectic. A similar observation could be made about the spectra of whole-rock compositions of aphyric lavas in other studies²².

I propose that the very existence of the pyroxene paradox in MORB glasses and aphyric lavas is evidence of the picritic nature of parental magmas for MORB. Despite the possible complications of crystal accumulation⁸, such parental magmas would have had compositions similar to those of the picritic lavas found at MORB sampling sites. Although it is difficult to specify the exact Mg content(s) of such picritic parental magmas, olivine megacrysts with forsterite contents reaching Fo 91 (ref. 3) require the existence of MORB liquids with at least 13–15 wt% MgO. A comparison of terrestrial picrite suites⁸ indicates that such MORB parental magmas are rich in Al but poor in Fe with respect to the parental magmas of other tholeiitic suites.

The results of the Baffin Bay study emphasize the importance of highly porphyritic lavas to the petrogenetic interpretation of volcanic suites. A rigorous evaluation of the implications of this study for MORB would require a detailed comparison of the compositions of glass and bulk lava in picritic samples. Unfortunately, these data are scarce as such rocks have commonly been avoided because they were considered to be 'cumulates'.

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Flock-feeding on fish schools increases individual success in gulls

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Flock-feeding and the role of white plumage in gulls and other seabirds have been the subject of much debate^{1–4}. At first sight it seems that competition within the flock would render flock formation against the interest of the bird who finds the fish school, as the fish must then be shared with birds joining the flock. However, it is also possible that flock formation is neutral or even beneficial to the individual members⁵, including the bird that found the fish^{4,6} (the 'first finder'). Here we show that the fishing success of individual black-headed gulls, *Larus ridibundus*, increases with flock size up to at least eight birds. Part of the reason is that the fish school is more vulnerable when attacked by several gulls. The first gull to reach a fish school therefore benefits from being joined by others, and conspicuous white upper parts in gulls may act as a means of attracting other gulls to the flock and hence improving hunting success.

Twelve black-headed gulls that had been caught in the wild (six adults and six yearlings, all in mainly white winter plumage) were monitored while hunting schooling bleak (*Alburnus alburnus*; length 6.5 ± 0.8 cm, mean $\pm$ s.d.) in a 5×5 m pool. The pool was situated in a 10×7 m room illuminated with 16 40-W fluorescent lights (at a height of 2.9 m above the pool). The pool was 14 cm deep with a light-grey bottom, and sloping at the edges over a width of 40 cm. This decrease in water depth at the periphery of the pool prevented the fish from hiding there.

Black-headed gulls are light (~ 275 g) and agile, hence they were able to manoeuvre easily in the room. They attacked the fish by plunging their heads under water; between attacks they flew or swam 1–3 m to locate new fish. The gulls became habituated to captivity within a few days and entered the room containing the pool to hunt when allowed to do so. For 5 days in each week, the gulls were allowed to hunt in groups of various sizes. At weekends, the birds were fed fish offal *ad libitum*. Gulls were tested singly, in flocks of three, and in flocks of six. Such triplets of tests were run 10 times for each gull; the combination of birds in each flock was chosen by drawing random numbers. Each hunting session lasted for 3 min.

On each trial day, each bird was tested in the morning and in the afternoon. After a test we re-stocked the pool with fish to maintain the number in the school at 350; this size of school covered an area of ~ 0.5 m². Handling time per fish was less than 1 s, and a gull usually caught 10–30 fish during a 3-min test. One hidden observer per gull noted each attempted and each successful attack using a tape recorder, as well as any period lasting ≥ 3 s during which the gull did not hunt (time spent searching for fish, preening or fighting). During some tests, the events in the central 6 m² of the pool were recorded using a video camera mounted beneath the ceiling.

During the entire 3½-week test period, the hunting success per gull was higher in triplets than in singletons, and higher in groups of six than in triplets (Fig. 1a; unless specified, we used two-tailed randomization tests for statistical comparisons⁷). Suc-

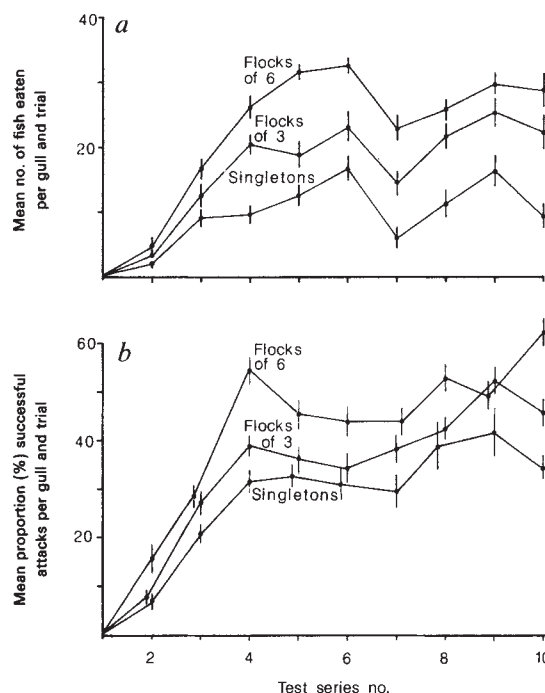


Fig. 1 a, The number of fish (mean $\pm$ s.e.m.) eaten per gull per 3-min trial in all test series was higher for flocks of 3 birds than for single gulls, and higher in flocks of 6 birds than in flocks of 3 birds. For test series 6–10, this trend was tested by calculating the linear regression of success in relation to flock size for every gull. The regression coefficient is positive for each of the 12 birds, with a mean of 3.18 ± 0.36 (significantly different from zero, $P = 0.0005$). The means are almost identical for adults and juveniles: 3.25 and 3.10, respectively. For each additional flock member, the hunting success per gull thus increases by an average of about three fish per 3-min hunting session. b, The proportions of successful attacks (mean $\pm$ s.e.m.) per gull per trial was also higher for flocks of 3 birds than for singletons, and higher in flocks of 6 birds than in flocks of 3 birds (with one exception). The trend in relation to flock size is statistically significant ($P = 0.001$; test as in a).

cess increased until the sixth series of tests. After this point, success was lower after each weekend (after series 6 and 9), probably because the gulls were satiated from eating *ad libitum*; they gained weight.

For test series 6–10, the catch per bird in flocks of six was 20–50% higher than in flocks of three birds and $>100\%$ higher than for singletons (Fig. 1a). The proportion of successful attacks per gull also increased with flock size (Fig. 1b); the averages were 34, 43 and 50% success in flocks of 1, 3 and 6 birds, respectively. Thus, on average, it was advantageous for the birds to fish in flocks.

To test directly whether the 'first finder' benefits from flock formation, we compared the success (number of fish caught) of each gull hunting as a singleton with its success as a flock member during the same test series. If every gull is better off as part of a flock than as a singleton, the first finder clearly benefits from being joined by other birds. We made this comparison for test series 6–10; the 12 gulls formed four flocks of three and two flocks of six per series. Of 20 possible comparisons for flocks of 3, each gull fared better as part of a flock than as a singleton in 14 cases; in 5 cases one gull fared worse in the flock, and in one case a gull had equal success as both a singleton and flock member. In all 10 comparisons for flocks of 6, the individual gulls fared better in the flock than as singletons. Although not amenable to statistical testing because of the dependence between the series, these results indicate that even the first finder benefits from flock formation.

During a 3-min trial, the birds hunted, on average, for 168, 172 and 175 s in flock sizes of 1, 3 and 6, respectively, which is

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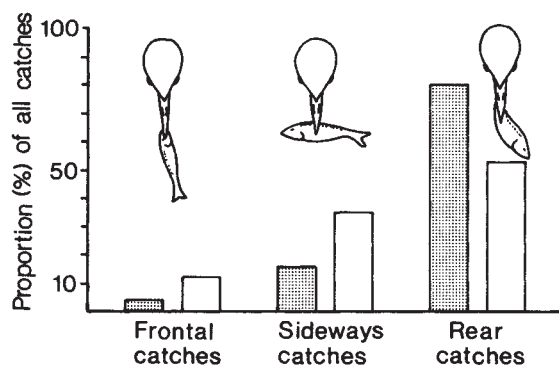


Fig. 2 Gulls in flocks (open bars) caught higher proportions of their prey from a frontal (12%) or sideways direction (35%) than did single gulls (4 and 16%; stippled bars), which captured most fish from behind ($P < 0.001$, χ^2 test, d.f. = 2). For each catch visible on the video films, we measured the direction of the plunging gull in relation to the school or solitary fish just prior to the catch. Three classes of directions (each 120° wide) were scored: frontal, which means the gull met the fish within $\pm 60^\circ$ of its forward direction; sideways, within $\pm 30^\circ$ of the right angle to either side of the fish; and rear, within $\pm 60^\circ$ of the tail direction of the fish. The direction was determined for 75 catches by single gulls, and for 313 catches by members of flocks of 6.

indicative of a slightly decreased period of vigilance in the larger flocks. To allow for this possibility, we calculated hunting success per s of foraging time; again, hunting success increased with flock size ($P = 0.0005$; test as in Fig. 1a).

Similar results were obtained in two previous test series with 6 and 8 gulls, hunting in flocks of up to 6 and 8 birds. Although these two series finally ran out of fish before hunting success reached a plateau, the success per gull increased with flock size ($P = 0.06$ and 0.02 , respectively). Because we monitored different individuals from those tested above, this result strengthens our conclusion and suggests that hunting success in this situation increases up to a flock size of at least eight.

The greater hunting success of gulls in flocks as determined here perhaps arose because they received more practice in flocks than alone (they were tested in flocks of two sizes, 3 and 6, for each run as a singleton). To examine this possibility, 3 weeks after the experiment we randomly split the 12 gulls into two groups of six. In 10 sessions, one group was then tested as a single flock of six; the other birds were tested individually. Again, hunting success was more than twice as high in the flock of 6 birds ($P = 0.04$, one-tailed test based on the 12 individual means). The trend in success with flock size therefore is not an effect of unequal practice.

There are several possible reasons for the higher hunting success of birds in flocks^{5,8}. The activity of other flock members might stimulate a bird to more intense efforts or guide it to prey sites⁹. If relevant, however, this is not the whole explanation: time spent resting, preening or searching for fish during the 3-min tests decreased ($P = 0.03$) from flock size 1 to 6 by an average of only 7.5 s. This difference accounts for only a small part of the increase in the total catch.

Another, probably more important reason is altered vulnerability of the fish. The video films showed that the fish school was split into smaller and looser groups by a flock than by a single gull. We counted the number of fish in each of 30 rectangles (40×45 cm), monitored by the camera during tests with single gulls and flocks of six. Each count was done at 60 s from the start of a test, given that $> 75\%$ of all fish were then visible; if not, samples were taken at 10-s intervals until this criterion was met. We then calculated the variance/mean ratio in number of fish per square. Random (Poisson) dispersion corresponds to a ratio of 1, clumping to a larger ratio. The average ratio with flocks of six was 14.1 (s.e. = 1.8; $n = 9$), with single gulls 28.4

(s.e. = 2.2; $n = 10$). Hence the fish became more dispersed when hunted by flocks ($P = 0.0002$). This should increase the vulnerability of the fish if, as seems likely in small forms, schooling is an adaptation that reduces predation^{10,11}.

The gulls caught more fish from a head-on or sideways direction when hunting in flocks than when alone (Fig. 2). Some fish that were being hunted were driven from one gull towards others. It should be harder to catch a fleeing fish than one that is approaching, which must turn to escape, or one on a lateral course, which presents a larger target surface area. Consistent with this idea, a larger proportion of frontal (46%) and sideways (50%) attacks were successful compared with attacks from the rear (38%) ($n = 736$, $P < 0.01$, χ^2 test, d.f. = 2; data from flocks of six). No coordination was apparent among the gulls, however, nor did several together chase single fish separated from the school, as do some predatory fish^{10,12}.

The total catch per gull rose with flock size owing to both a higher rate and a higher success of attacks. With the rate of attacks held constant at singleton level, the increase predicted from higher success alone accounted for 30% (s.d. = 23%, range 6–76%, $n = 12$) of the observed increase, while a higher attack rate (success instead held constant) accounted for 51% (s.d. = 22%, range 18–100%) of the increase.

Seabirds in the wild often encounter hunting conditions similar to those of the present experiment when schools of fish are driven to the surface by predatory fish or marine mammals^{11,13,14}. As flock size becomes large, any benefits of flock fishing may be partly offset by reduced success, owing, for example, to rapid diving of the fish. However, schools can remain near the surface for several minutes, even if attacked by hundreds of birds¹³. The present trials, of 3 min duration, therefore seem realistic.

White plumage can make a bird easier to discern^{15,16}. Larids are often the first to find fish schools, and conspicuous white gulls and terns attract more birds^{9,11,13,17}. Our results suggest that not only the 'joiners' but also the first finder benefit from formation of a flock. Hence, conspicuous white or contrasting plumage in larids and other seabirds may provide a means, favoured by selection among individuals, of attracting other birds and hence improving hunting success. Whereas white underparts may also provide hunting camouflage^{2,4,16,18}, this probably is not the case for white upper parts; advantages of group foraging seem a more likely explanation.

The present results have a bearing on another aspect of foraging in seabirds. The 'information centre' hypothesis suggests that a main advantage of colony formation is that unsuccessful foragers can follow others to rich food sources^{19,20}. Possible evidence in support of this hypothesis is that birds often leave colonies together, but they may do so also for other reasons^{17,20–22}. If, as our results indicate, a gull that finds a fish school benefits from being joined by other birds, gulls should depart and forage together. Thus, seabirds following a 'leader' from a colony need not be heading for any particular site, but may go along simply because group foraging is beneficial. This could explain why flock leaders call more often than followers²².

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Vernier acuity of neurones in cat visual cortex

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The ability of human observers to detect Vernier breaks of as little as 5 s of arc has been termed hyperacuity¹ as this distance is substantially less than the angular separation of the bars of the highest spatial frequency of grating (~1 arc min) that can be detected. Although the visual cortex is a likely candidate for the location of detectors involved in this performance², it is not known whether there are cells sensitive enough to detect deviations from co-linearity that are small compared with their spatial resolution (defined in terms of the highest spatial frequency that the cell can detect). We report here the results of physiological experiments on single units in area 17 of the cat visual cortex in which we studied the effect of introducing a Vernier break into a bar stimulus moved across the receptive field of the cell at a constant velocity. Our results show that the responses of most simple and complex cells are significantly reduced by the introduction of a Vernier break that is substantially smaller than the spatial resolution of the cell. The most sensitive cells in our sample could discriminate Vernier offsets of 3-6 arc min with a reliability of ~70%. This was much smaller than their spatial resolution, which was in the range 25-30 arc min. We interpret these results in terms of mechanisms that could underly the orientation selectivity of cortical neurones and suggest how our results relate to human Vernier acuity.

We made recordings with extracellular platinum-iridium micro-electrodes in area 17 of the cortex of 13 normal adult cats. Anaesthesia was induced with intravenous pentothal and subsequently maintained by respiration with 70% N₂O and 30% O₂. The animals were paralysed with Flaxedil, and the eyes were refracted using contact lenses with 3 mm artificial pupils onto a tangent screen 137 cm away. Two moving-bar stimuli, one from each of two computer-controlled optic benches, were projected onto the screen³. Each bar was typically between 1/10

and 1/4° wide and 1 and 2° long. The bars were oriented parallel with each other at the preferred orientation of the cell, and were normally placed end to end with no intervening gap. The total length of the bars was usually about the same as the length of the receptive field. In most experiments we moved the bars across the receptive field in the preferred direction of the cell and at a constant velocity close to the preferred velocity of the cell, normally between 0.5 and 3° s⁻¹. The stimuli were presented monocularly to the dominant eye. Vernier offsets of from -30 arc min to +30 arc min in 6-arc min steps were introduced, providing 11 different stimulus conditions; in a further 2 conditions each bar moved on its own through the receptive field. We normally tried to centre the stimulus in the receptive field so that each of the two bars moving on its own produced a similar response, although precise centering turned out to be unimportant. Responses were averaged over 8-16 presentations of each particular stimulus, given in random order. We often made other quantitative measurements, including a line-weighting function and orientation- and velocity-tuning curves. We measured spatial resolution either by listening to an audiometer or by examining computer-averaged responses from the cell as high-contrast grating stimuli of varied spatial frequency were moved across the receptive field. The bars of the gratings were the same length as that of the stimulus used to determine the Vernier acuity of the cell. All the cells in our sample had receptive fields that lay 3-15° from the area centralis. Of the 82 cells studied, 27 were classified as simple and 47 as complex; 8 could not be classified.

In most cells, the response, measured either in terms of peak firing rate or as total number of spikes per presentation, was reduced by the presence of a Vernier break (Figs 1, 2). Simple cells did not seem to differ from complex cells in this respect, and were among the most and the least sensitive cells in our sample. We found examples of cells with Vernier sensitivity in all laminae. Typically, responses were reduced by 20-50% of maximum for a Vernier offset of 30 arc min, although in the most sensitive cells the average response could be halved by a change in Vernier offset of 6 arc min.

We used an approach similar to that recently adopted by Parker and Hawken⁴ to estimate the ability of a single neurone reliably to signal information about the presence of a Vernier offset. We compared the responses of a cell (measured in terms of the total number of impulses evoked) to presentations of a pair of stimuli differing in Vernier offset by a given amount. A correct response was scored if the responses of the cell differed in the expected direction, that is, if the response was greater for a smaller Vernier offset. An incorrect response was scored if the response was in the opposite direction. This comparison was carried out for all pairs of presentations in a series that took from 5 to 10 min to complete. As a means of describing the Vernier acuity of a cell this method has the advantage of taking into account the reliability of the response to a change in Vernier offset. This could be a better way to describe its performance than measuring the slope of the Vernier tuning curve averaged over many presentations.

Fig. 1 A, a-h, Impulse frequency histograms from an experiment on a complex cell located in layer V of area 17; the cell had a receptive field ~3° from the area centralis. **B**, The stimulus configuration and the shape of the receptive field are drawn to scale relative to the location of the area centralis (cross). Arrow, direction of movement of the stimulus; bars are offset by ~6 arc min. Responses **a-h** are to Vernier offsets of -18, -12, -6, 0, 6, 12, 18 and 24 arc min, respectively. Each histogram shows the response to a single presentation of the stimulus. The presentations were in random order and made every 4 s. The velocity of the bars was 1° s⁻¹. Scale bars: **A**, 500 s⁻¹ (vertical); 500 ms (horizontal). **B**, 1°.

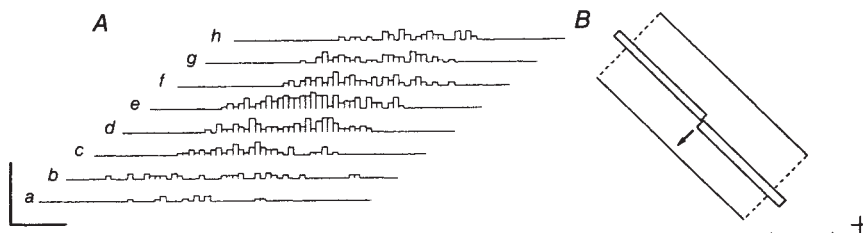
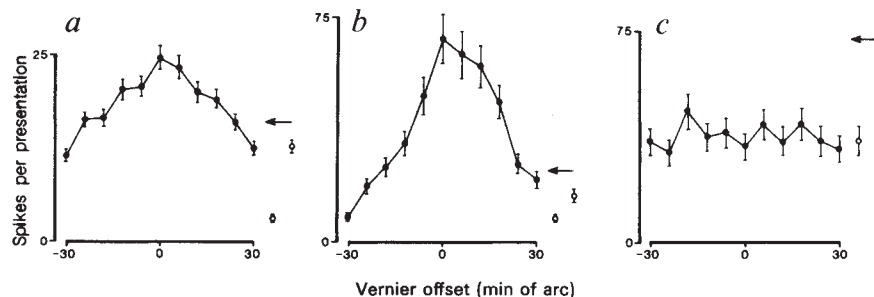


Fig. 2 Total number of impulses evoked plotted against Vernier offset for three different units. *a*, Simple; *b*, *c*, complex. The final two points in each graph (open circles) are the response to each bar presented separately. Arrow, linear sum of these responses. *a*, From a layer IV simple cell with an odd symmetrical receptive field 5° from the area centralis. The receptive field is 2° across, each bar is $0.2 \times 1.1^\circ$, each response is the average of 16 presentations. *b*, From a complex cell located at the layer V/VI boundary; *c*, from a complex cell located in the upper layers of area 17 with a receptive field 10° from the area centralis. Each response is the average of eight presentations. Standard error bars are shown.



This method of analysis showed that many cells could discriminate 6–12 arc min of Vernier offset with $>70\%$ reliability. Some cells did better than this (Fig. 3); the most sensitive cell in our sample (Figs 1, 3) achieved 88% correct performance for a 6 arc min Vernier offset, implying a Vernier threshold of ~ 3 arc min for a 70% criterion of reliability. Figure 3 also shows one other such Vernier-discrimination curve from a cell that could detect 6 arc min of Vernier offset with 77% reliability. The spatial resolution of this cell was measured and found to be between 25 and 30 arc min. Its Vernier discrimination is thus an example of neuronal hyperacuity.

Inhibitory and, to a more variable extent, excitatory interactions between the component bars of the stimulus seem to be involved in producing these effects. In cells with good Vernier tuning, the response to the bars in the aligned condition was often greater than the sum of the responses to each bar alone (Fig. 2*a*, *b*). As Vernier offset was increased, the response decreased to a value less than this sum. The changeover from excitation to inhibition occurred typically for offsets of ~ 12 –24 arc min. In other cells, including both simple and complex

types, length summation was approximately linear, although the cells nevertheless showed good Vernier tuning. The reduction in the response caused by Vernier offset in these cells was presumably entirely caused by inhibition.

In a few cells (2 simple and 14 complex) the response was unaffected by the presence of a Vernier break. In these units, the response to the bars in the aligned condition was always similar to the response to each bar on its own (Fig. 2*c*). It was never possible to induce Vernier sensitivity in these cells by changing the stimulus properties, for example, bar length, separation, contrast or brightness of the stimulus. We recorded several cells showing some intermediate degree of Vernier sensitivity, so it could be that there are not two distinct classes of Vernier sensitivity, but rather a continuum.

We found evidence that the interaction producing the Vernier sensitivity has features in common with that responsible for the orientation selectivity of the cell. A Vernier stimulus can be approximated by a single bar of the same overall length, with an orientation the same as a line connecting the midpoints of the two component bars (Fig. 4*c*). Two observations suggest

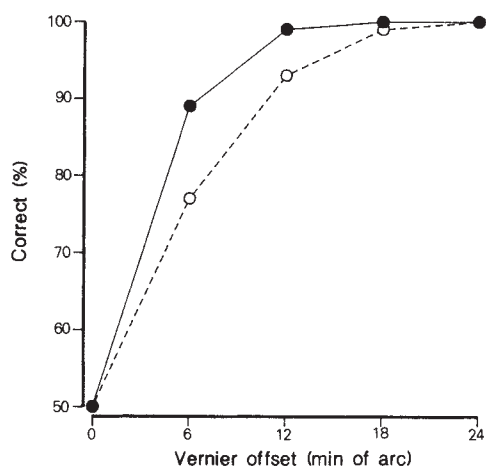


Fig. 3 Correct discriminations (%) plotted against Vernier offset for two of the most sensitive cells in our sample. A percentage discrimination of 70% for a Vernier offset of 6 arc min means that on 70% of presentations differing in Vernier offset by 6 arc min (for example, stimuli with offsets of 12 and 18 arc min), the response of the cell, measured in terms of the total number of impulses evoked, was greater for the smaller Vernier offset. Each point is based on many comparisons, for example, an experiment in which a set of nine Vernier offsets differing in 6-arc min steps were presented 16 times would yield $(9-1) \times 16^2 = 2,048$ comparisons for the 6 arc min point on the graph. Filled circles, results from the cell shown in Fig. 1; open circles, results from a complex cell in layer III.

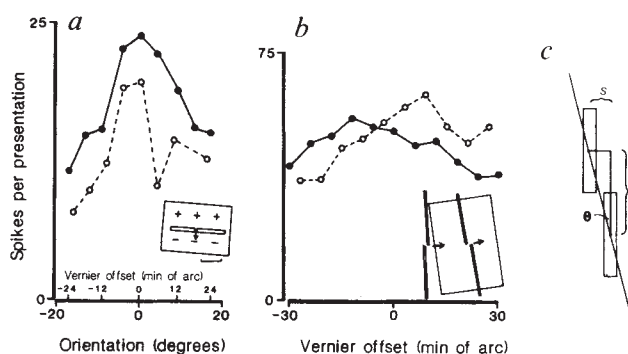


Fig. 4 *a*, Comparison between a Vernier tuning curve (filled circles) with Vernier offset re-expressed as equivalent orientation (defined in *c*), and an orientation-tuning curve (open circles) obtained for a bar of the same overall length as the Vernier stimulus. The sizes of the receptive field and the stimulus are shown. The difference in the height of the two curves is probably caused by a change in the responsiveness of the cell between experiments. Scale bar, 1° . *b*, Effect of changing the overall orientation of the Vernier stimulus on the tuning curve. Filled and open circles are for stimuli (drawn to scale relative to the receptive field size) whose overall orientation differed by 8° . The separation between the bar centres is 2.6° ; the change in the orientation of the stimulus is 8° so the tuning curve should shift by $2.6^\circ \tan(8^\circ) = 22$ arc min, which is close to the shift actually observed. *c*, The definition of equivalent orientation used here; the equivalent orientation of two bars whose centres are separated by a distance d , and with a Vernier offset of s , is $\theta = \tan^{-1}(s/d)$.

that cells were treating both the Vernier stimulus and this approximation to it as equivalent. First, in several cells for which we made a quantitative measure of the orientation tuning curve (using a bar of the same overall length as the Vernier stimulus), re-expressing the Vernier tuning in terms of an equivalent orientation tuning gave a curve that was comparable in shape (Fig. 4a). This relationship was found to hold true for cells with both poor and good Vernier tuning. Second, changing the overall orientation of the Vernier stimulus shifted the position of the peak of the Vernier tuning curve (Fig. 4b). The size of this shift, re-expressed as an equivalent orientation, matched the change in the overall orientation of the stimulus.

Our results may be relevant to an understanding of Vernier acuity as measured in psychophysical experiments. Although other factors are probably involved, detection of orientation differences close to the Vernier break seems to be an important cue in Vernier tasks^{5,6}. Thus, detection of Vernier offset, alignment of dots along a vertical axis, curvature or chevron distortions all have thresholds that, expressed in terms of the orientation cues available, are comparable with the ability to detect the deviation of a straight line from vertical^{7,8}. This angle varies from $\sim 1/3$ to 1° (depending to some extent on the skill of the observer)⁷. Because the orientation-tuning curves of cortical cells (in monkeys) have a half-width at half-height of rather more than this ($\sim 5^\circ$), Westheimer¹ concluded that they cannot be involved in hyperacuity-type tasks. However it is arguable that the output from any kind of detector whose response is a smoothly varying function of the position or configuration of a retinal stimulus and is sufficiently noise-free, could, in principle, serve as a basis for hyperacuity-type discrimination. Thus, if two stimuli merely give rise to a statistically significant difference in the firing rate of a neurone they could, as a direct consequence, be discriminable in a behavioural task.

If this is true then a signal from a cortical cell with an orientation-tuning curve with a half-width at half-height of 5° might well subserve an orientation discrimination of $\leq 1^\circ$. This performance could be improved on if the outputs from several cells with similar properties were averaged, although the number of cells over which an average of this sort could be taken is uncertain. Parker and Hawken⁴ have recently studied the performance of neurones in monkey visual cortex and found cells with resolution and contrast sensitivity thresholds, and a predicted sensitivity to positional displacement in the hyperacuity range which is similar to that shown behaviourally by monkeys. This is evidence that the nervous system either does not average the outputs of several cortical neurones to improve its performance, or that an averaged response is degraded by noise elsewhere in the nervous system, perhaps at the level of some subsequent decision-making process. The ability of the nervous system to interpret fractional changes in the firing rates of neurones in this way, either above threshold (as we have suggested) or close to it (as Parker and Hawken⁴ suggest) would eliminate the need for a fine-grain representation of positional information as hypothesized elsewhere^{9,10}.

Our present data show that some cells, recorded at a retinal eccentricity of $3-5^\circ$, were able to discriminate $3-6$ arc min of Vernier offset with $\geq 70\%$ reliability. This was substantially less than their spatial resolution, which lay in the range $25-30$ arc min. It is also less than the best resolution of retinal ganglion cells at this eccentricity, which is in the range of $15-25$ arc min (ref. 11), and limits the maximum possible resolution of cortical neurones to which they provide inputs. One can extrapolate from these data to estimate the likely Vernier acuity of cells with receptive fields in the area centralis, where spatial resolution is about half that at the eccentricity from which our data came¹¹. On the basis that Vernier acuity increases in proportion to resolution, some cells with receptive fields in the area centralis could be expected to have Vernier acuities as high as 1.5 arc min. This is better than the threshold of 5 arc min measured in behavioural experiments by Berkley and Sprague²;

however, a different behavioural method shows a threshold of 1.2 arc min, which is similar to the postulated neural threshold (K. Murphy and D. E. Mitchell, personal communication). When compared with a behavioural grating resolution of $\sim 7-8$ arc min (ref. 12), the behavioural Vernier acuity of the cat is, like that of humans, well within the hyperacuity range. Berkley and Sprague's finding² (confirmed by Mitchell and Murphy) that lesions in area 17, but not in other visual areas, drastically reduce Vernier acuity, also supports the notion that the performance of cells with properties similar to those we have studied could be the limiting factor in the achievement of a high Vernier acuity in both cats and humans.

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Reciprocal changes in corticotropin-releasing factor (CRF)-like immunoreactivity and CRF receptors in cerebral cortex of Alzheimer's disease

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Alzheimer's disease is a progressive degenerative disease of the nervous system characterized neuropathologically by the presence of senile plaques and neurofibrillary tangles in amygdala, hippocampus and neocortex¹⁻⁴. Dysfunction and death of basal forebrain cholinergic neurones projecting to forebrain targets^{5,6} are associated with marked decreases in cholinergic markers, including the activity of choline acetyltransferase (ChAT)^{2,3,7-9}. Although cortical levels of somatostatin¹⁰⁻¹² and somatostatin receptors¹³ are reduced in Alzheimer's, no consistent changes have been reported in other neuropeptide systems^{12,14-17}. We have now examined in control and Alzheimer's brain tissues pre- and postsynaptic markers of corticotropin-releasing factor (CRF), a hypothalamic peptide regulating pituitary-adrenocortical secretion^{18,19} which also seems to act as a neurotransmitter in the central nervous system (CNS) (reviewed in refs 20, 21). We have found that in Alzheimer's, the concentrations of CRF-like immunoreactivity (CRF-IR) are reduced and that there are reciprocal increases in CRF receptor binding in affected cortical areas. These changes are significantly correlated with decrements in ChAT activity. Our results strongly support a neurotransmitter role for CRF in brain and demonstrate, for the first time, a modulation of CNS CRF receptors associated with altered CRF content. These observations further suggest a possible role for CRF in the pathophysiology of the dementia. Future therapies directed at increasing CRF levels in brain may prove useful for treatment.

Brains were obtained post mortem from 14 individuals with Alzheimer's disease and 11 neurologically normal controls (Table 1). One cerebral hemisphere was chosen at random for neurochemical study, the other was placed in formalin for quantitative neuropathological analysis. Standard areas of frontal pole, temporal pole, occipital pole and cingulate cortex (directly above the genu of the corpus callosum) were subdivided from the fresh brain, frozen in dry ice and stored (-70°C) until they were processed for CRF radioimmunoassay²², CRF receptor binding studies and ChAT assays²³. Formalin-fixed samples of the cortex and hippocampus were embedded in paraffin, cut ($10\mu\text{m}$) and stained with haematoxylin/eosin and silver impregnation methods. All Alzheimer's cases showed abundant neuritic plaques (>15 plaques per low-power field) and neurofibrillary tangles; the control cases showed no evidence of pathology in the brain.

Table 1 Age, sex distribution, postmortem delay and drug treatment data of controls and individuals with Alzheimer's disease

	Controls	Alzheimer's patients
Sex	7 male; 4 female	8 male; 6 female
Age (yr)	64.4 ± 6.4 (39-96)	71.6 ± 2.1 (57-84)
Postmortem delay (h)	10.5 ± 2.2 (0.5-18)	8.0 ± 1.3 (1-16)
Drug treatment	No. of patients	
No medication	11	6
Neuroleptics	0	3
Antidepressants	0	1
Neuroleptic/antidepressant	0	1
Anti-anxiety agents	0	1
Neuroleptic/anti-anxiety agents	0	2

Values for age and postmortem delay are mean $\pm$ s.e.m. with the range of values in parentheses. No significant differences were found between control and Alzheimer's groups.

Previous studies have defined the characteristics and distribution of CRF receptors in rat²⁴ and human²⁵ pituitary glands and in rat brain^{26,27}. Here we have used a stable ^{125}I -labelled tyrosine extended analogue of rat/human CRF, ^{125}I -Tyr⁰-rat/human CRF (NEN), to study the characteristics of the CRF binding site in human cerebral cortex. The binding was saturable and of high affinity, with an apparent dissociation constant (K_D) of $5.5 \pm 0.4\text{ nM}$ and maximum binding (B_{max}) of $170 \pm 6\text{ fmol per mg protein}$ (mean $\pm$ s.e.m.; $n = 3$). In competition studies, ovine CRF was equipotent with rat/human CRF in inhibiting ^{125}I -Tyr⁰-rat/human CRF binding, with an inhibitor affinity constant (K_i) of $2.4 \pm 0.2\text{ nM}$. The weak receptor antagonist α -helical CRF(9-41) was less potent (K_i 59.4 nM), and the rat/human CRF fragment, CRF(21-41), which is a very weak stimulator of proopiomelanocortin-derived peptide secretion, displaced only 20% of the specific binding at $1\mu\text{M}$ concentration. At comparable $1\mu\text{M}$ concentrations, the rat/human CRF-related fragments CRF(1-20)OH and CRF(6-33) and the unrelated peptides arginine vasopressin and angiotensin II did not inhibit ^{125}I -Tyr⁰-rat/human CRF binding. The kinetics and pharmacological properties of ^{125}I -Tyr⁰-rat/human CRF binding sites in human cerebral cortex are comparable to those for the biologically relevant CRF receptor in rat^{24,28} and human²⁵ pituitary and rat brain^{26,27,29}.

In individuals with Alzheimer's, the concentration of CRF-IR (Fig. 1a) were decreased and CRF receptors (Fig. 1b) were reciprocally increased in the temporal, frontal and occipital cortex. The changes in CRF activity were greatest in the occipital cortex, with an 80% decrease in the content and a doubling in receptor binding; the reductions in CRF-IR and increases in

CRF receptors in the temporal and frontal cortex were all greater than 50% of the corresponding control value. Neither CRF-IR nor CRF receptors were changed significantly in the cingulate cortex. Scatchard analysis data of detailed competition curves carried out in random samples of temporal, frontal and occipital cortex from both control and Alzheimer's disease patients indicate that the increase in ^{125}I -Tyr⁰-rat/human CRF receptor binding reflects an up-regulation in the receptor number (B_{max}), as the affinity of the ligand for the receptor (K_D) was comparable in control and Alzheimer's groups in all the cortical areas examined. A highly significant negative correlation ($r = -0.65$; $P < 0.005$) existed between the change in CRF receptors and CRF-IR in each cortical area.

To examine possible interactions between cholinergic deficits in Alzheimer's disease and alterations in CRF markers, ChAT activity was correlated with CRF-IR and with CRF receptors in each cortical area. Significant positive relationships existed between ChAT activity and CRF-IR levels in the frontal ($r = +0.68$; $P < 0.05$), temporal ($r = +0.79$; $P < 0.05$) and occipital ($r = +0.74$; $P < 0.01$) cortex, and significant negative correlations were detected between ChAT activity and CRF receptors in that temporal ($r = -0.61$; $P < 0.05$) and occipital ($r = -0.77$; $P < 0.01$) cortex. In contrast, in both the control and AD groups, neither the age nor sex of the patient nor the postmortem interval correlated significantly with CRF-IR, CRF receptors or ChAT activity in any of the cortical regions examined. In addition, no effect of any psychoactive drug medication was noted on CRF receptors in Alzheimer's patients whereas a statistically non-significant trend towards a small increase in CRF-IR was noted in those patients treated with neuroleptics.

In rodents, CRF neurones are intrinsic to the neocortex, with perikarya located in laminae II and III and terminal fields concentrated in laminae I and IV (refs 30, 31), areas rich in CRF receptors^{26,27}. CRF neurones probably have a similar distribution in the human cortex. Our finding of a decrease in CRF-IR in the frontal and temporal cortex in Alzheimer's disease is similar to that observed previously³². The reduction in CRF-IR in the cerebral cortex in Alzheimer's patients could result from a selective degeneration of the intrinsic cortical CRF neurones and/or disease of CRF neurones projecting to the cortex from other brain areas. For example, a subpopulation of brain stem cholinergic neurones containing CRF project to various forebrain targets including the cortex³³, and pathology of this system or other CRF-cholinergic neurones innervating forebrain targets could reduce ChAT and CRF-IR. Decreased CRF content in these systems could result from cell dysfunction, that is, decreased synthesis, altered processing, increased degradation, increased release and/or cell death. Increased CRF release appears unlikely, as it might be expected to down-regulate the CRF receptors rather than produce the observed up-regulation. At present, we cannot identify the specific mechanisms leading to reduced CRF levels. Alterations in CRF receptors in the cortex in Alzheimer's disease contrast with previous observations of changes in the cholinergic and somatostatin systems in which reductions in presynaptic cholinergic markers^{2,3,5-9} and somatostatin content¹⁰⁻¹² are accompanied by inconsistent changes in cholinergic receptors³⁴⁻³⁶ and decreased somatostatin¹³ receptors. The up-regulation of cortical CRF receptors in Alzheimer's disease under conditions in which the endogenous peptide is reduced is, to date, a unique finding in the study of neuropeptides in disease, and suggests that the CRF-receptive cells may be preserved in the cortex in Alzheimer's disease. We are now investigating the immunohistochemical localization of CRF and the autoradiographic distribution of CRF receptors in normal human cortex, and the relationship of CRF neurones to the classical features of Alzheimer's disease.

The cerebral cortical cholinergic deficiency seems to be the most severe and consistent neurochemical deficit^{3,6,7,37,38}. The demonstration of significant correlations between changes in

CRF and ChAT activity in human cortex, complemented by similar preliminary studies in rodents^{33,39}, suggest that the CRF and cholinergic systems interact. As mentioned above, some brain stem cholinergic neurones projecting to the frontal cortex also contain CRF³³. In behavioural studies, CRF injected into the rat frontal cortex does not initiate, but significantly inhibits the effects of carbachol, a muscarinic cholinergic receptor agon-

ist, on 'boxing' behaviour³⁹, suggesting that CRF may exert a neuromodulatory function on cholinergic systems. CRF appears to interact with somatostatin, another neuropeptide affected by Alzheimer's disease¹⁰⁻¹², in that it stimulates the somatostatin secretion from dispersed rat cerebral cortical cells⁴⁰. Additional studies are necessary to define the functional importance of these cholinergic-peptidergic and peptidergic-peptidergic interactions in the pathophysiology of the disease.

Thus, CRF-IR is significantly reduced in the cortex of individuals with Alzheimer's disease and there are reciprocal increases in CRF receptor binding. The demonstration of an up-regulation of CRF receptors following a decrease in CRF-IR indicates a physiological relevance of the receptor site and is consistent with the concept that CRF acts as a neurotransmitter in normal cortical functions and that disease of this peptidergic system may be important in certain clinical manifestations of the disease.

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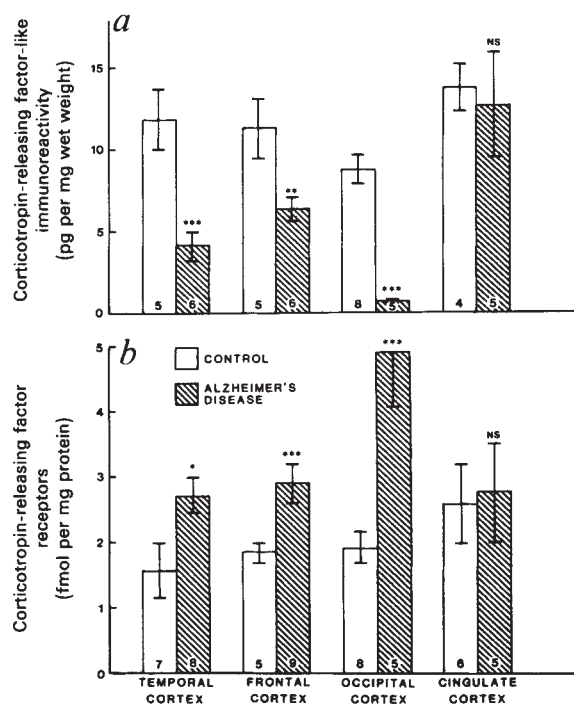


Fig. 1 CRF-like immunoreactivity (a) and CRF receptor binding (b) in discrete regions of the cerebral cortex of Alzheimer's patients and controls. All values are means $\pm$ s.e.m. The number of subjects in each group is given at the bottom of each histogram. Data were analysed for differences using a Student's *t*-test. Significant differences from control group at $P < 0.05$, $P < 0.025$ and $P < 0.005$ are denoted by *, ** and ***, respectively. NS, not significant.

Methods: a, Autopsy samples were weighed, extracted in hot 1 M acetic acid/0.1 M HCl, centrifuged and then the supernatants defatted three times with 4 vols ether:petroleum ether, 1:2. The aqueous phases were applied to a BondElut C₁₈ cartridge and the CRF eluted and lyophilized as described previously²². Reconstituted samples were assayed using HPLC-purified ¹²⁵I-labelled rat/human CRF analogue substituted with norleucine at amino acid residues 21 and 38, ¹²⁵I (Nle^{21,38})-rat/human CRF tracer, rabbit anti-rat/human CRF serum (C-68²²) and rat/human CRF as a standard. Results are expressed as pg CRF-like immunoreactivity per mg wet weight. b, For receptor binding studies in membrane homogenates, regions of cerebral cortex were weighed and homogenized using a Teflon pestle in 10 vol ice-cold 50 mM Tris-HCl, 10 mM MgCl₂, 2 mM EGTA and 0.32 M sucrose, pH 7.2. The homogenate was centrifuged at 4°C for 10 min at 1,000g. The resulting supernatant was recentrifuged at 4°C for 30 min at 20,000g and the pellet was resuspended in 50 mM Tris-HCl, 10 mM MgCl₂, 2 mM EGTA, 0.1% bovine serum albumin, aprotinin (100 kIU ml⁻¹) and 0.1 mM bacitracin, pH 7.2, at a final protein concentration of 0.3–0.6 mg ml⁻¹. The binding of ¹²⁵I-Tyr⁰-rat/human CRF (NEN; 2,200 Ci mmol⁻¹) to CRF receptors was carried out in 1.5-ml polypropylene micro-tubes in a final volume of 300 μ l with 30–60 μ g protein and 40,000 c.p.m. ¹²⁵I-Tyr⁰-rat/human CRF. Nonspecific binding was determined in the presence of 10⁻⁶ M rat/human CRF (Peninsula). The reaction was allowed to proceed for 2 h at room temperature. The tissue was separated from the solution by centrifugation in a Beckman Microfuge for 3 min at 12,000g. The resulting pellet was washed gently with 1 ml of phosphate-buffered saline, pH 7.4, containing 0.01% Triton-X, and the contents were recentrifuged for 3 min at 12,000g. The supernatant was aspirated and the radioactivity measured in a γ -counter at 80% efficiency.

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Na-Ca exchange current in mammalian heart cells

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Electrogenic Na-Ca exchange has been known to act in the cardiac sarcolemma as a major mechanism for extruding Ca ions¹⁻³. Ionic flux measurements in cardiac vesicles have recently suggested that the exchange ratio is probably 3 Na:1 Ca (refs 4, 5), although a membrane current generated by such a process has not been isolated. Using the intracellular perfusion technique^{6,7} combined with the whole-cell voltage clamp⁸, we were able to load Na⁺ inside and Ca²⁺ outside the single ventricular cells of the guinea pig and have succeeded in recording an outward Na-Ca exchange current while blocking most other membrane currents. The current is voltage-dependent, blocked by La³⁺ and does not develop in the absence of intracellular free Ca²⁺. This report presents the first direct measurement of the cardiac Na-Ca exchange current, and should facilitate the study of Ca²⁺ fluxes during cardiac activity, together with various electrical changes attributable to the Na-Ca exchange⁹ and the testing of proposed models¹⁰⁻¹².

Single ventricular cells were dissociated from guinea pig hearts using collagenase. A whole-cell voltage-clamp experiment was performed using a wide-tipped (4-5 µm) glass pipette (resistance 1-2 MΩ) which facilitated the diffusion of the pipette solution into the cell. The compositions of the bath and the pipette solutions were designed to block most of the channel currents and the electrogenic Na-K pump, so that time-dependent current components largely disappeared and the current-voltage (*I-V*) relations obtained using ramp pulses became almost linear (Fig. 1B). The cell was initially perfused with 0 Na⁺ internal solution and Ca²⁺-free external solution, and in these conditions, superfusing Ca²⁺ (1 mM) slightly shifted the membrane current outward (Fig. 1A). The *I-V* curves measured before (Fig. 1Ba) and during (b) Ca²⁺ application illustrate that external Ca²⁺ (Ca_o²⁺) in the absence of internal Na⁺ (Na_i⁺) did not induce a significant current change. After washing off Ca_o²⁺, the pipette solution was changed into one containing 30 mM Na⁺, which had very little effect on the membrane conductance (Fig. 1Cc, d). Subsequent superfusion of 1 mM Ca²⁺ in the presence of Na_i⁺ induced a marked outward shift of the holding current. Washing off Ca_o²⁺ quickly turned off the current. The *I-V* relation taken at the peak effect (Fig. 1Ce) revealed a significant voltage dependence of the outward current; thus, the current was larger at more positive potentials and was almost exponential in the range between -120 and +60 mV. In the presence of 0.1 mM La³⁺, which is known to block Na-Ca exchange, 1 mM Ca_o²⁺ did not activate the current (Fig. 1A). The magnitude of the current became larger when a higher concentration of Ca_o²⁺ was perfused and it became smaller when a lower concentration of Na⁺ was in the pipette, indicating that the current depends on both Na⁺ and Ca²⁺ concentrations. These results suggest that the current is generated by the exchange of internal Na⁺ for external Ca²⁺.

The Na-Ca exchange current is driven by the concentration gradients of both Na⁺ and Ca²⁺ across the membrane. If the exchange ratio is 3 Na:1 Ca, as has been suggested by others^{4,5}, the reversal potential of the Na-Ca exchange current (E_{NaCa}) should be given by the following thermodynamic equation³:

$$E_{\text{NaCa}} = 3E_{\text{Na}} - 2E_{\text{Ca}}$$

where E_{Na} and E_{Ca} are the respective reversal potentials for Na⁺ and Ca²⁺. If the ionic conditions are 140 mM Na_o⁺, 30 mM Na_i⁺, 1 mM Ca_o²⁺ and 73 nM Ca_i²⁺, E_{NaCa} will be -131 mV. This value is strikingly close to the potential at which the *I-V* curves before (d) and in the presence of 1 mM Ca_o²⁺ (e) are about to

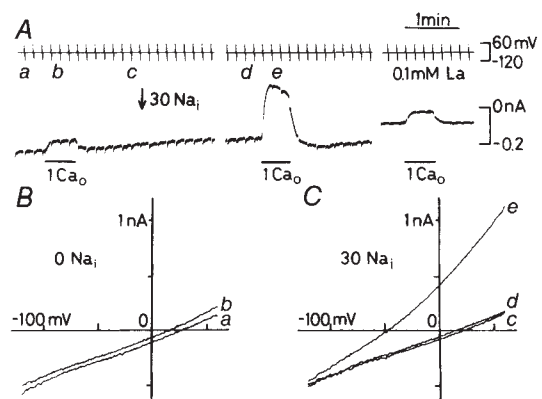


Fig. 1 A, Voltage-clamp records from a single myocyte showing voltage (upper trace) and current (lower trace). A ramp pulse to +60 mV and then to -120 mV was given from the holding potential of -30 mV every 10 s at a ramp speed of about 0.2 V s⁻¹. The *I-V* curves were obtained from the descending portion of this pulse from +60 mV to -120 mV. To exchange the pipette solution, a piece of tapered polyethylene tube was inserted in the pipette, through which a new solution was introduced within 1 min by applying a negative pressure inside the pipette. The arrow indicates when the solution exchange was started. The holding current shifted slightly outward on loading Na⁺. The middle record shows 1 mM Ca_o²⁺ superfusion in the presence of Na_i⁺. The Ca_o²⁺-induced outward current appears to decay after reaching a peak. Current undershoot is seen on washing off Ca_o²⁺. These phenomena may be due to Ca_o²⁺ accumulation immediately below the membrane or to acid induced by binding of Ca_o²⁺ to EGTA. The Ca_o²⁺-activated current did not show a significant time dependence. The last chart record starts at 3 min after applying 0.1 mM LaCl₃ in the external solution. La³⁺ shifted the holding current outward and reduced the slope conductance of the background current. The *I-V* relation (not shown) taken during Ca_o²⁺ superfusion revealed about 90% inhibition of the Ca_o²⁺-induced current by La³⁺. The interruptions between the panels were 7 min on the left and 5 min on the right. Points a-e are described in parts B and C. B, *I-V* relations measured before (a) and during (b) 1 mM Ca_o²⁺ superfusion in the absence of Na_i⁺. C, *I-V* curves before (c) and after (d) loading Na_i⁺ and during subsequent 1 mM Ca_o²⁺ application (e). The membrane capacitance of this cell was 180 pF. A specific membrane capacitance was assumed to be 1 µF cm⁻² to estimate the membrane area.

Methods. The external solution (34-35 °C) was Ca²⁺-free, K⁺-free Tyrode's solution containing (in mM) NaCl 140, MgCl₂ 2, HEPES 5 (pH 7.4) and the following current blockers: 1 mM BaCl₂ and 2 mM CsCl to block K channels, 2 µM D600 for Ca channels and 20 µM ouabain to block the Na-K pump. The control pipette solution contained 126 mM Cs⁺ to replace internal K⁺, 42 mM aspartic acid, 42 mM EGTA and the free Ca²⁺ was adjusted to pCa 7.1 (73 nM) by adding 21 mM CaCl₂. The stability constant of EGTA of 1.55×10^7 (pH 7.4) was used¹⁵. The high concentration of EGTA was to prevent the cell from contracture by Ca_o²⁺ influx. To make a Na⁺-containing pipette solution, Na⁺ was replaced by equimolar Cs⁺. It also contained (in mM) Mg-ATP 10, MgCl₂ 3, creatine phosphate 5, tetraethylammonium (TEA) 20 to block the K channel and 5 HEPES-CsOH buffer (pH 7.4).

meet in Fig. 1C. No straightforward contribution of ionic currents could give this reversal potential.

Before concluding that this current is the Na-Ca exchange current, we investigated whether the current possessed a unique property found in squid axon, that is, whether the Na-Ca exchanger requires Ca_i²⁺ for its activation. This feature was described by Baker and McNaughton¹³, who injected EGTA into squid axon and observed the inhibition of Ca_o²⁺-dependent Na⁺ efflux. More recently, Allen and Baker¹⁴ confirmed this result by injecting Quin-2, another Ca chelator, into squid axon. Figure 2 illustrates that superfusion of the ventricular cell with 1 mM Ca_o²⁺ in the absence of Ca_i²⁺ (42 mM EGTA pipette solution) failed to induce an outward current even in the presence of 30 mM Na⁺ in the pipette. When the internal solution

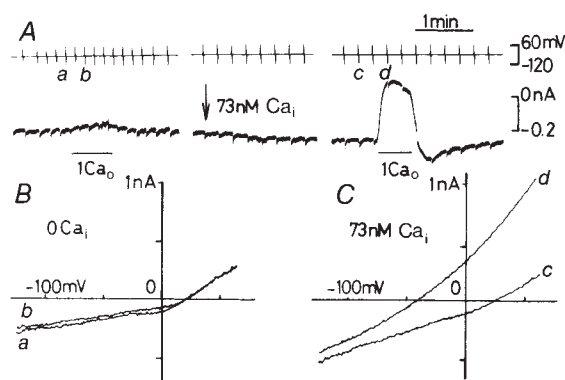


Fig. 2 **A**, Chart record of voltage (upper trace) and current (lower trace). Ramp pulses of the same shape as described in Fig. 1 were given from -30 mV every 10 or 15 s. The left panel shows the application of 1 mM Ca_0^{2+} in the absence of Ca_i^{2+} . After a 1-min interruption in the middle panel, the pipette solution was changed into one containing 73 nM Ca_i^{2+} (at arrow). In the right panel, after a 5-min interruption, 1 mM Ca_0^{2+} superfusion induced a large outward current. **B**, I - V curves before (a) and during (b) superfusion of 1 mM Ca_0^{2+} in the absence of Ca_i^{2+} . The Ca current is not completely blocked and the remaining component can be seen as a slight inward deflection at around 0 mV. **C**, I - V relation in the presence of 73 nM Ca_i^{2+} before (c) and during (d) 1 mM Ca_0^{2+} superfusion. The capacitance of this cell was 246 pF.

was changed to one containing 73 nM Ca_i^{2+} (42 mM EGTA and 21 mM CaCl_2), the membrane conductance increased slightly. The subsequent superfusion of 1 mM Ca_0^{2+} then induced a large outward current. Reapplying Ca_0^{2+} after returning to the Ca_i^{2+} -free pipette solution did not induce the current. This result not only confirms that the current is generated by the Na-Ca exchange but also indicates that the requirement for Ca_i^{2+} is a general property of the Na-Ca exchanger.

The amount of Ca_i^{2+} influx calculated from the current at 0 mV (Figs 1C, 2C) is ~ 28 pM $\text{cm}^{-2} \text{ s}^{-1}$, a larger value than that reported for squid axon³ (0.5 pM $\text{cm}^{-2} \text{ s}^{-1}$ at 100 mV driving force) in different ionic conditions. The slope conductance of this current is ~ 23 $\mu\text{S cm}^{-2}$ in the above ionic environment. In physiological conditions, the steady-state membrane conductance is about $1,300$ $\mu\text{S cm}^{-2}$ at the resting potential and about 20 $\mu\text{S cm}^{-2}$ at plateau potentials⁷. The Na-Ca exchange current may therefore influence the plateau of the action potential more significantly than it does the resting potential. There is a strong possibility that the Na-Ca exchange current is involved in the arrhythmogenic potential oscillation that appears when the heart cells are overloaded with Ca^{2+} . More information about intracellular Na^+ and Ca^{2+} concentration dynamics is required to discover exactly how this current behaves during cardiac activity.

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Identification of Na-Ca exchange current in single cardiac myocytes

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In cardiac muscle the exchange of intracellular Ca^{2+} for extracellular Na^+ is an important transport mechanism for regulation of the intracellular free Ca^{2+} concentration ($[\text{Ca}]_i$) and hence the contractile strength of the heart (ref. 1; for reviews see refs 2-4). Due to its stoichiometry of $\geq 3:1$ $\text{Na}^+/\text{Ca}^{2+}$ (refs 3, 5), Na-Ca exchange is supposed to generate a current across the cell membrane. It is thought that such a current may contribute to cardiac action potential and physiological or pathological pacemaker activity^{6,7}. Although the occurrence of Na-Ca exchange is well documented, a membrane current generated by this transport has not been identified unequivocally. Previous attempts to detect such a current in multicellular preparations, for example, by measuring small current differences after varying the extracellular ionic composition⁸, although providing evidence, did not rule out other possible interpretations. Here we demonstrate that a transient rise in $[\text{Ca}]_i$ caused by release of Ca from sarcoplasmic reticulum (SR) generates a membrane current in cardiac myocytes. The dependence of this current on the transmembrane gradients for Na^+ and Ca^{2+} and on membrane potential meets the criteria for a current produced by electrogenic Na-Ca exchange. Cyclic activation of this current by release of Ca from the SR can cause maintained spontaneous activity, suggesting that Na-Ca exchange contributes to certain forms of cardiac pacemaking.

Figure 1 shows inward current transients (I_{ti}) recorded from an atrial 'cardioball' at constant membrane potential (-70 mV). In the experimental conditions described in Fig. 1 legend these current transients occurred regularly for periods of up to 50 min after the start of dialysis of a cell. (If the EGTA concentration in the dialysing fluid was raised to ≥ 1 mM, I_{ti} did not occur spontaneously, but could be evoked by a long-lasting or repetitive activation of voltage-dependent Ca current.) Extracellular application of caffeine (5 mM), a substance known to release Ca ions from, and to prevent their re-accumulating into, the SR of cardiac and skeletal muscle^{9,10}, evoked an inward current shift of almost identical amplitude (see ref. 11). In the presence of caffeine, I_{ti} was no longer observed (Fig. 1a). This result clearly suggests that I_{ti} reflects a rise in $[\text{Ca}]_i$ due to cyclic release of Ca ions from the SR¹². This conclusion is supported by the observation that I_{ti} is accompanied by visible contractions of the myocytes at constant negative membrane potential. In addition to the shift of the holding current, caffeine induced openings of an ionic channel with a large conductance (220 - 270 pS) which is not the elementary unit of the inward current shift¹³. Channel openings of this type can be detected occasionally also during spontaneous I_{ti} (Figs 1b, 2a).

With increasing depolarization, I_{ti} was decreased in amplitude and prolonged (Fig. 1c). After a step change into a voltage range more positive than -50 mV, only a single I_{ti} was observed, the relaxation of which was incomplete—that is, after a release event the current remained at a level which was negative with regard to the instantaneous current measured on the step depolarization. Relaxation of I_{ti} occurred only after repolarization to a more negative membrane potential. The range of membrane potential at which I_{ti} failed to relax was -52 to -38 mV (mean = -45 mV) in 22 cells loaded with 20 mM Na^+ . If the solution in the pipette contained 12 mM Na^+ the range was -34 to -17 mV (mean = -20 mV; 19 cells). This difference indicates an effect of intracellular Na or the transmembrane Na^+ gradient on the voltage dependence of I_{ti} .

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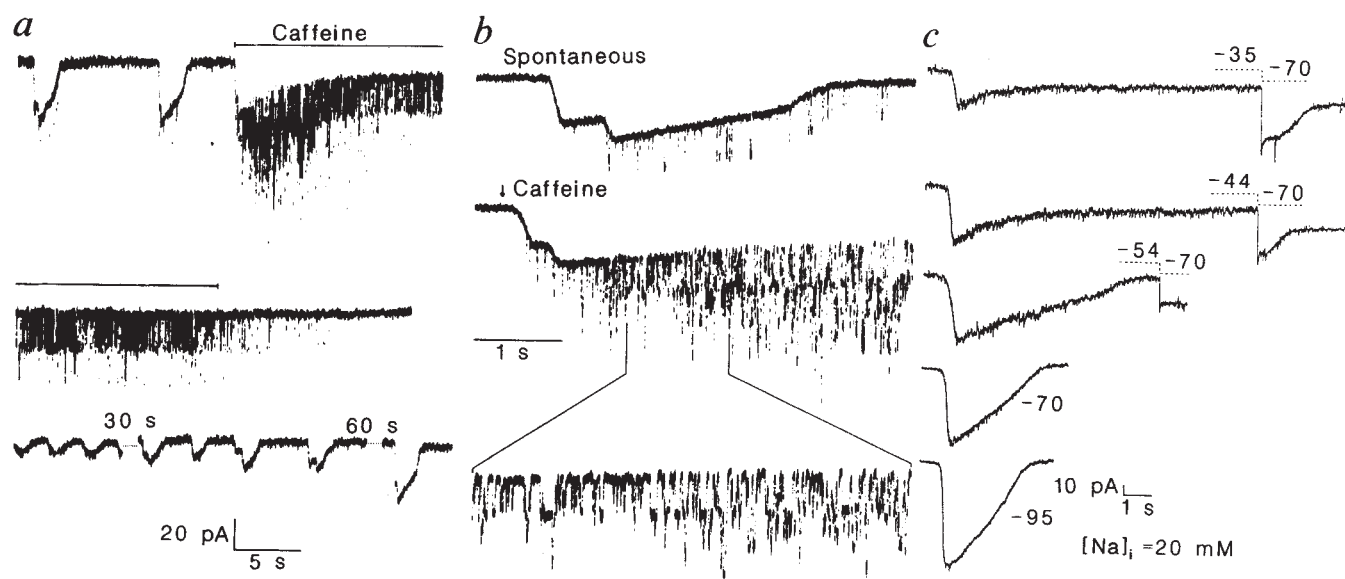


Fig. 1 *a*, Spontaneous transient inward current (I_{ti}) at constant membrane potential (-70 mV). Traces, from top to bottom, represent consecutive current recordings (interrupted at the times indicated by the gaps). The cell was dialysed with pipette-filling solution containing 12 mM Na^+ for ~ 10 min. That the voltage control was adequate was routinely tested by depolarizing clamp pulses into the voltage range of activation of the fast Na^+ current (I_{Na}). Voltage control was assumed to be adequate only if I_{Na} activation near threshold occurred without a distinct delay. Caffeine was applied for the time indicated, by pressure from a glass capillary (tip diameter ~ 5 μm) which contained the drug (5 mM) dissolved in extracellular solution. The tip of the capillary was placed ~ 10 μm from the surface of the cell. *b*, Spontaneous and caffeine-evoked current transient on an expanded timescale. *c*, Dependence of I_{ti} on membrane potential. From the holding potential (-70 mV), long-lasting changes of membrane potential were applied to the levels indicated. Note the transient inward current after repolarization from -35 and -44 mV but not from -54 mV.

Methods. Single cells were isolated from atria of adult guinea pig hearts by enzyme perfusion using collagenase and elastase. The cells were cultured for 3–14 days. In these conditions, spherical myocytes ('cardioballs') with a diameter of 15 – 25 μm are obtained¹⁸. Voltage-clamp experiments were performed using patch-clamp pipettes in the whole-cell recording configuration^{19,20}; this allows high-resolution current recording and internal dialysis. The pipettes were filled with a solution containing (in mM): Cs-citrate 65 , NaCl 20 , Mg-ATP 1 – 2 , cyclic AMP 0.1 , EGTA 0.05 ; HEPES/CsOH 10 , pH 7.2 . In some experiments the solution also contained 12 mM NaCl and 8 mM CsCl. Pipette resistance, 2 – 5 M Ω . The external solution had the following composition (mM): NaCl 140 , CsCl 2.0 , MgCl_2 1.0 , CaCl_2 2.0 , HEPES/NaOH 10 , pH 7.4 . Experiments were performed at room temperature (21 – 23 $^{\circ}\text{C}$). The current was measured by means of a patch-clamp amplifier (List LM/EPC 7), stored on analog tape and analysed later on an IBM PC at appropriate sampling rates.

Since, as demonstrated by the action of caffeine, I_{ti} is primarily caused by a rise in $[\text{Ca}]_i$, it can be concluded from Fig. 1c that the decay of $[\text{Ca}]_i$ after a release is slowed by membrane depolarization. $[\text{Ca}]_i$ does not decay to its resting level at membrane potentials less negative than -45 mV (20 mM $[\text{Na}]_i$) or -20 mV (12 mM $[\text{Na}]_i$). Thus, I_{ti} is related to a voltage-dependent process of Ca extrusion from the cytoplasm. The most likely candidate for such a voltage-dependent Ca extrusion is trans-sarcolemmal Na–Ca exchange, a transport system which uses the inwardly directed Na gradient as an energy source for outward transport of Ca^{2+} (refs 1–5). The reversal potential of this transport (E_{NaCa}) is given by^{2,3}

$$E_{\text{NaCa}} = (nE_{\text{Na}} - 2E_{\text{Ca}})/(n - 2) \quad (1)$$

where E_{Na} and E_{Ca} are the Nernst potentials for the respective ions, and n denotes the coupling ratio, which is likely to be $3:1$ $\text{Na}^+/\text{Ca}^{2+}$ (ref. 5). The resulting driving force is given by the difference between E_{NaCa} and E_m (the actual membrane potential). From this simple relation, it follows that for a given $[\text{Ca}]_i$ which is large enough to activate Na–Ca exchange¹⁴, a change in the driving force of either of the ion species involved should be equivalent to a corresponding change in membrane potential with regard to its effect on Na–Ca exchange: increasing the extracellular Ca concentration ($[\text{Ca}]_o$) or decreasing the gradient for Na^+ should affect the current in the same way as a membrane depolarization. Assuming a $3:1$ stoichiometry, an increase of the Ca concentration in the external solution from 2 to 5 mM should be equivalent to a depolarization of 23 mV. Figure 2a shows a representative experiment in which super-

fusion of the cell with a solution containing 5 mM CaCl_2 caused a shift of the holding current (at -70 mV) in the outward direction. The spontaneous I_{ti} was immediately reduced from 32 pA to 12 pA and was prolonged by ~ 3 s. A reduction of the extracellular Na concentration had a qualitatively similar effect (Fig. 2b): in this cell, which was loaded with 20 mM Na^+ , a depolarization from -80 to -60 mV caused a slower but still complete relaxation of I_{ti} . Incomplete relaxation was observed at -40 mV. A decrease of $[\text{Na}]_o$ from 140 to 100 mM, which should be equivalent to a depolarization of 25 mV, resulted in a decreased and prolonged I_{ti} at -80 mV. Incomplete relaxation of I_{ti} was observed at -60 mV. At -100 mV, current transients with a time course similar to those observed at -80 mV at normal $[\text{Na}]_o$ were recorded. Thus, the decrease in $[\text{Na}]_o$ shifted the voltage dependence of I_{ti} by at least 20 mV.

These results show for the first time that release of calcium from SR activates a membrane current which is related to Ca removal from the cell. This current depends on membrane potential and on the transmembrane gradients for Na and Ca ions in a manner expected for electrogenic Na–Ca exchange. In the present study we have not considered the kinetic aspects of this transport. In a recent investigation, an outward current caused by Na–Ca exchange was identified in Na-loaded myocytes, and this current did not develop in the absence of intracellular Ca ions—it was not determined solely by the driving forces of the ions involved¹⁴.

Transient inward currents due to oscillatory Ca release from SR have been described previously in Ca-overloaded multicellular preparations^{15,16}. Both electrogenic Na–Ca exchange and

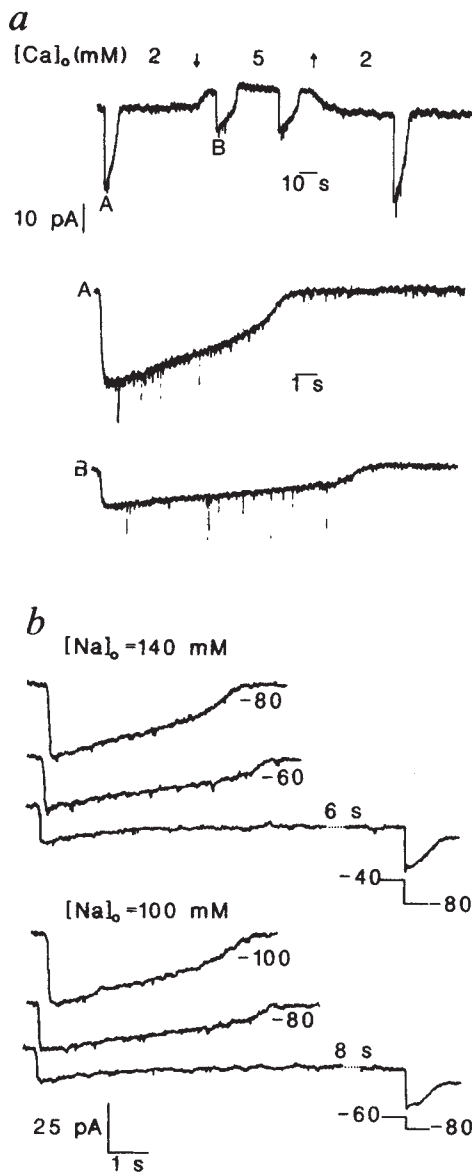


Fig. 2 Effect of changes in transmembrane Na^+ and Ca^{2+} gradients on spontaneous I_{ti} . *a*, Effect of increase in $[\text{Ca}]_o$ on spontaneous transient inward current. During the time indicated by the arrows the cell was superfused (see Fig. 1 legend) with a solution containing 5 mM CaCl_2 . Note the openings (A and B) of the large-conductance channel, which are inadequately resolved at this time resolution. *b*, Effect of a reduction of $[\text{Na}]_o$ on I_{ti} . The upper three traces represent inward currents recorded in a solution containing 140 mM Na^+ at the membrane potentials indicated (mV). The lower traces were recorded during superfusion with a solution containing 100 mM NaCl , 40 mM being substituted by LiCl . After initiation of superfusion with the low-Na solution, the holding current was shifted in the outward direction by 16 pA.

a Ca-activated nonspecific cation channel have been suggested as charge-carrying mechanisms. The latter interpretation was supported by the discovery of a Ca-activated nonspecific cation channel in the membrane of cultured neonatal myocytes¹⁷. From the results of the present study, the major mechanism behind I_{ti} is clearly a Na-Ca exchange current. Activation of a Ca-dependent ion channel could explain neither the parallel shift of the voltage dependence of I_{ti} by 20 mV on decreasing $[\text{Na}]_o$ from 140 to 100 mM, nor the effect of an increase in $[\text{Ca}]_o$ (Fig. 2). The single-channel events detected occasionally after spontaneous Ca release and which are more pronounced after

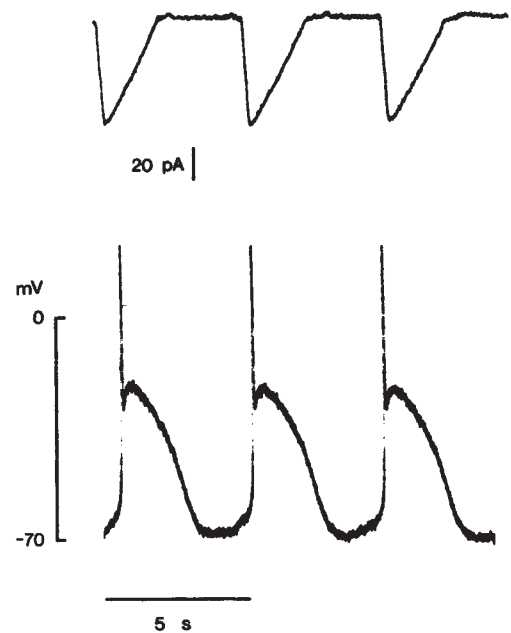


Fig. 3 Spontaneous electrical activity due to cyclic I_{ti} . The top trace shows the membrane current recorded from a cell held at a membrane potential of -70 mV (voltage clamp). The bottom trace was recorded from the same cell under current clamp conditions. To obtain a negative 'diastolic potential', a constant hyperpolarizing current of 22 pA was injected.

release of Ca from the SR by caffeine (see Fig. 1) point to a possible contribution of gated channels to I_{ti} in multicellular preparations. Note, however, that the channel found in the present study is unlikely to be identical to the nonspecific channel described previously¹⁷, which has a much smaller unit conductance.

A possible contribution of Na-Ca exchange to cardiac electrical activity is suggested by the result in Fig. 3. Spontaneous I_{ti} causes rhythmic action potentials consisting of a fast overshooting (Na^+) 'spike' and a long-lasting plateau. Although this action potential configuration is far different from any physiological one, this result indicates a role for Na-Ca exchange current in cardiac excitation.

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Repetitive transient rises in cytoplasmic free calcium in hormone-stimulated hepatocytes

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In the stressed animal, the vasoactive hormones vasopressin and angiotensin-II and the neurotransmitter noradrenaline induce liver cells to release glucose from glycogen. The intracellular signal that links the cell-surface receptors for noradrenaline (α_1) and vasoactive peptides to activation of glycogenolysis is known to be a rise in the cytoplasmic concentration of free calcium ions (free Ca^{2+})¹⁻³. The receptors for these agonists induce the hydrolysis of phosphatidylinositol 4,5-bisphosphate, a minor plasmalemma lipid, to produce inositol trisphosphate and diacylglycerol³⁻⁵. Inositol trisphosphate has been shown to mobilize intracellular calcium in hepatocytes^{3,6-8}. We show here, by means of aequorin measurements in single, isolated rat hepatocytes, that the free Ca response to these agonists consists of a series of transients. Each transient rose within 3 s to a peak free Ca of at least 600 nM and had a duration of approximately 7 s. The transients were repeated at intervals of 0.3-4 min, depending on agonist concentration. Between transients, free Ca returned to the resting level of ~200 nM. Clearly, the mechanisms controlling free Ca in hepatocytes are more complex than hitherto suspected.

Hepatocytes were isolated from fed, mature male rats and were kept at 37 °C in a complete culture medium for use within

10 h. Cells of about 25 μm diameter were injected with aequorin (see Fig. 1 legend). Stable resting signals could be recorded from individual hepatocytes for several hours. The mean resting signal for 55 cells was 1.2 photon counts s^{-1} above background (0.8 or 1.6 c.p.s.) and mean total counts per cell 2.6×10^5 . These measurements give an upper estimate for mean resting free Ca of 211 ± 9 ($\pm$ s.e.m.) nM ($n = 55$), assuming 1 mM free Mg and uniform calcium distribution. Arsenazo(III) and quin2 measurements also give values of ~200 nM (refs 1, 2).

The α_1 -preferring adrenergic agonist phenylephrine induced repetitive transient rises in free Ca which usually peaked at above 600 nM and were blocked reversibly by phentolamine, an α_1 -antagonist (Fig. 1a). Propranolol, a β -adrenergic antagonist (2 μM), had no effect on transients induced by 2 μM phenylephrine, while yohimbine, an α_2 -preferring antagonist (2 μM), reduced the frequency of transients induced by 2 μM phenylephrine (the period rose from 40 s to 60 s; data not shown). Only a proportion of the hepatocytes responded to phenylephrine (34/42 = 81%), a rather higher percentage than respond to α_1 -adrenergic stimulation by entering into DNA synthesis (~30%)⁹. The frequency of the transients was dependent on the concentration of phenylephrine (Fig. 1b). Low concentrations (0.6 μM), which activate phosphorylase by ~10% of maximum¹, induced transients at ~100-s intervals, while 2 μM phenylephrine produced transients every 20-30 s (Fig. 1b). At the higher concentrations (2-10 μM), the initial few transients were usually of higher magnitude and more frequent than those later in the series, when the period stabilized at ~30 s. At very high phenylephrine concentrations (100-1,000 μM), free Ca briefly reached 1,100 nM and fell slowly over ~2 min before repetitive transients appeared with a period of ~20 s (Fig. 1c). Adrenaline induced similar response patterns but was more potent (0.3 μM adrenaline was comparable to 2 μM phenylephrine; data not shown).

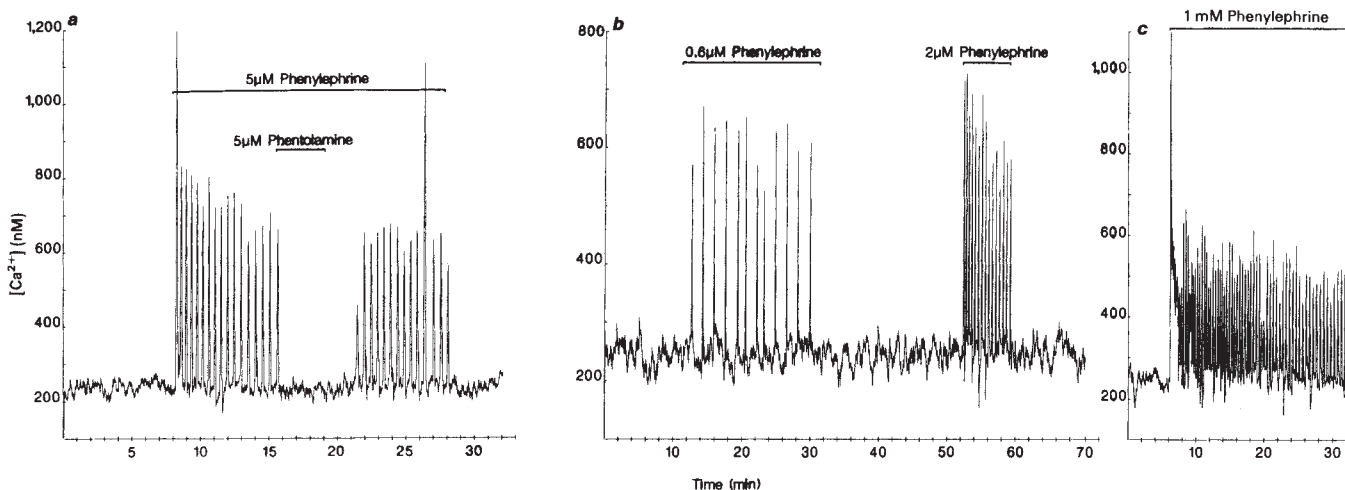


Fig. 1 Aequorin signals, transformed by microcomputer into free Ca values (nM), from individual rat hepatocytes exposed to phenylephrine. **a**, Phentolamine (5 μM) reversibly blocked free Ca transients induced by 5 μM phenylephrine. **b**, The frequency of the transients was dependent on the concentration (0.6 and 2 μM) of phenylephrine. **c**, Very high concentrations of phenylephrine (1 mM) generated a slow-falling rise, briefly reaching ~1,200 nM free Ca (peak not shown), followed by transients. (Time constants were 15 s for resting levels and 1 s for the transients.) **Methods.** Hepatocytes were prepared by perfusion of collagenase²⁴ (Boehringer) through livers from fed, male rats (150-250 g) and kept at 37 °C in CO_2 -bicarbonate-buffered Williams' medium E (Flow Labs) containing 1.8 mM Ca, 0.05% bovine serum albumin but lacking insulin or serum. The aequorin technique was modified from refs 25 and 26 as follows. Single hepatocytes of ~25 μm diameter, selected under a $\times 180$ stereomicroscope for their healthy appearance (no blebs, no vacuolation, distinct nuclei), were transferred to a 0.1-mm microslide (Camlab) containing a pool of 1% agarose (Sigma VII) and held at 37 °C under a layer of liquid paraffin. The cell was positioned within 100 μm of the agarose-oil interface before gelling the agarose at 4 °C for 2 min. Cells were injected on the stage of an inverted microscope ($\times 600$, Nomarski) at ~35 °C with aequorin solution from a micropipette whose tip had been filled with a 50- μm long column of aequorin. (The tips of freshly pulled pipettes when immersed in liquid paraffin for a few seconds will subsequently allow a short column of aequorin solution to enter spontaneously.) Injected cells that had retained the distinct concave appearance of their nuclei were transferred in the microslide to a perfusable cup held at ~37 °C positioned under a low-noise photomultiplier²⁵. Photon counts were sampled every 50 ms by a Sirius microcomputer. Data were plotted using exponential smoothing with time constants as indicated and calibration as before²⁵, except that 1 mM free Mg^{2+} was used.

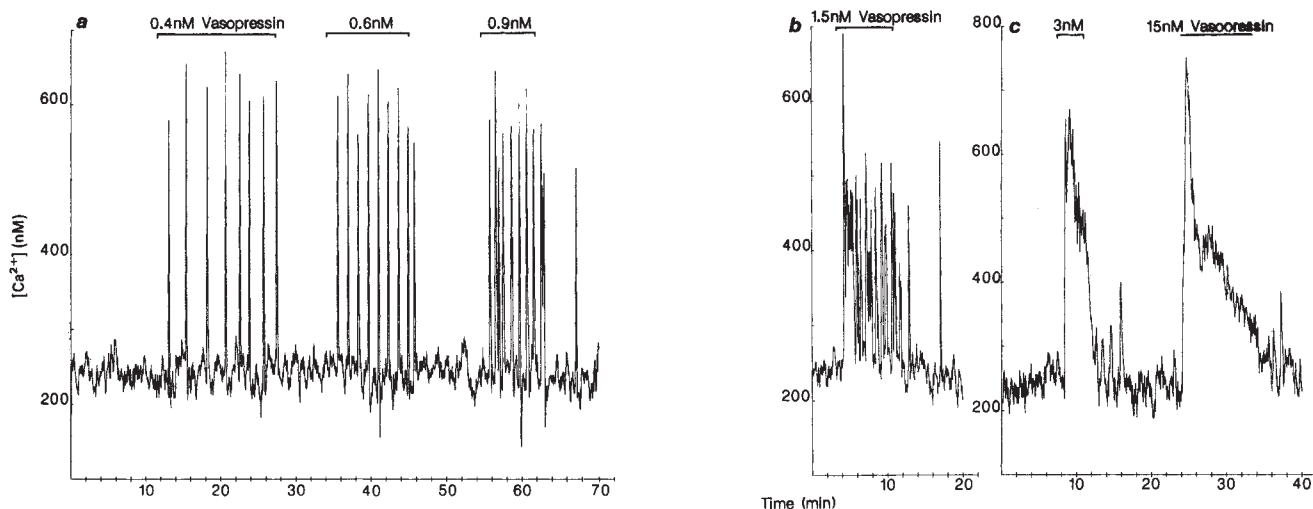


Fig. 2 Aequorin signals plotted as free Ca (nM) from a single hepatocyte exposed to various concentrations of $[Arg^8]$ vasopressin (time constants as Fig. 1). *a*, Physiological concentrations of vasopressin (0.4, 0.6, 0.9 nM) induced free Ca transients at increasing frequencies (periods were 120, 80 and 60 s, respectively). Before the 0.4 nM recording, the cell had failed to generate transients when exposed to 0.2 nM vasopressin for 8.5 min. *b, c*, High concentrations of vasopressin (1.5, 3 and 15 nM) induced a more sustained initial free Ca rise. Repetitive transients occurred in 1.5 nM, but not at the higher concentrations until the hormone was removed (time constants in *c* were 10 and 3 s).

Vasopressin and angiotensin-II at concentrations of ~ 1 nM, comparable to the K_m for activation of glycogen phosphorylase^{10,11}, induced a series of free Ca transients whose frequency was again dependent on hormone concentration (Fig. 2*a*). The onset of low-frequency transients (period 1–4 min) was preceded by a lag comparable to the period. Lags have been reported previously in free Ca measurements on populations of hepatocytes^{2,3,12}. Very high concentrations of peptide hormones (≥ 10 nM), which would induce measurable degradation of phosphatidylinositol 4,5-bisphosphate and receptor occupancy^{13,14}, induced a more sustained, slowly decaying rise in free Ca which peaked at 600–800 nM (Fig. 2*c*). The time course of these sustained free Ca responses is similar to that of changes in phosphatidylinositol levels and phosphorylase activation described at high hormone doses^{13,14}. At intermediate vasopressin concentrations (1.5 nM; Fig. 2*b*), an initial sustained rise in free Ca was followed by transients, similar to the response to very high doses of phenylephrine (Fig. 1*c*). Most cells responded to vasopressin (32/34), including 5 cells that had failed to respond to phenylephrine. Angiotensin induced a response in 21/27 cells (78%).

Figure 3 shows that the rate of rise of free Ca in a transient was rapid, taking ~ 2 –3 s to rise from resting levels (200 nM) to peak values (800–1,200 nM). The peak concentration was sustained for ~ 2 s, while the fall to resting levels took a further 3–5 s. Overall duration was ~ 7 s. No appreciable change in these parameters was detected at different hormone concentrations. Although quin2 measurements on populations of hepatocytes^{2,3} have not revealed transient behaviour, perhaps because of buffering of free Ca or poor cell synchrony, oscillations have been detected in the extracellular calcium concentration in the perfusate from intact liver exposed to 10 μ M phenylephrine (H. Sies, personal communication).

It is an attractive possibility that the frequency of the transients determines the intensity of a cell's response to hormone concentrations within the physiological range. Calcium concentrations within the range we observe can activate phosphorylase kinase^{15,16}, which contains covalently bound calmodulin. This kinase in turn activates glycogen phosphorylase, whose activity will be a function of the rates of phosphorylation and dephosphorylation. Since the frequency of the transients is the only parameter of free Ca which varies with hormone concentration, it is possible that frequency determines the rate of glycogenolysis in an individual cell. Rasmussen and Barrett have postulated

that a transient rise in free Ca to micromolar levels followed by a fall to ~ 500 nM would initiate and sustain calmodulin activity without promoting mitochondrial calcium overload¹⁷. In hepatocytes, the free Ca response is more complex than this, as free Ca falls to resting levels between transients, but our data offer a plausible explanation for the lack of mitochondrial calcium overload in chronically stimulated cells. Campbell has proposed that calcium acts as an intracellular regulator for 'threshold phenomena' that are either 'switched on' or 'switched off'¹⁸. Each free Ca transient conforms to this concept, while the occurrence of frequency-modulated series of transients may allow the amplitude of the glycogenolytic response in a single cell to be varied according to hormone concentration. There are indications from blowfly salivary glands¹⁹, pancreatic islet β -cells²⁰ and oocytes during fertilization^{21–23} that the phenomenon

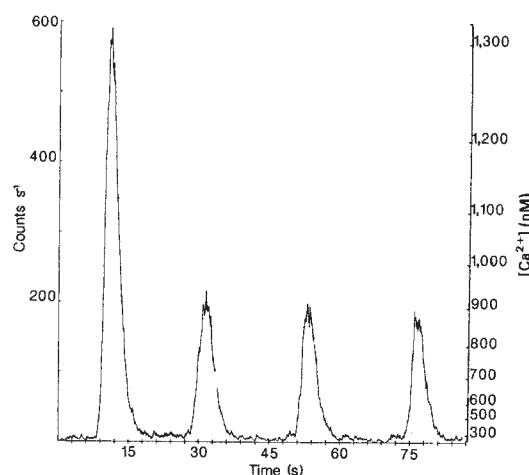


Fig. 3 The aequorin signal, expressed as photoelectron counts s^{-1} and as free Ca (nM), from a single hepatocyte responding to 5 μ M phenylephrine. Time constant, 0.5 s. The signal rose from resting to peak values within 2–3 s. Owing to dead space in the perfusion chamber, the exact time of arrival of phenylephrine at the cell was unknown. The background was 1.6 counts s^{-1} and the resting level 2.4 counts s^{-1} above background, corresponding to 200 nM free Ca. The cell contained a total of 4.2×10^5 photoelectron counts.

of repetitive transient rises in free Ca may be widespread in cells that use free Ca as a messenger for extracellular ligands.

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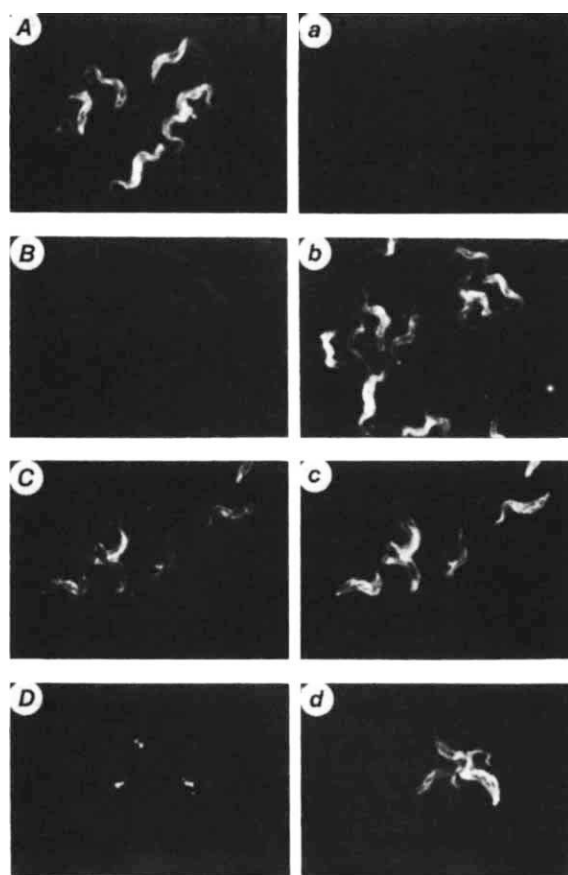


Fig. 1 Co-expression of two VSGs by cloned *T. equiperdum*. *A-D*, Fluorescence fluorescence (revealing VSG-1); *a-d*, Texas red fluorescence (VSG-201). Trypanosomes were washed in phosphate saline glucose buffer pH 8 (PSG)¹¹, deposited on microscope slides, air-dried and fixed for 1 min in 4% formaldehyde in phosphate-buffered saline (PBS) and for 2 min in acetone. After washing in PBS, the fixed trypanosomes were incubated for 30 min at room temperature in a 1:1 mixture of monospecific rabbit anti-VSG-1 (diluted 1:500 in PBS) and monoclonal mouse anti-VSG-201 (undiluted culture supernatant). After washing with PBS, bound antibodies were revealed with a 1:1 mixture of Texas red conjugated sheep anti-mouse immunoglobulin (Amersham, diluted 1:50 in PBS) and fluorescein-conjugated goat anti-rabbit immunoglobulin (Nordic Immunologicals, diluted 1:100 in PBS). The slides were washed and examined using appropriate filters with fluorescent illumination. *A, a*, Pure BoTat-1; *B, b*, pure BoTat-201; *C, c*, double expressors; *D, d*, live trypanosomes expressing VSG-1 and VSG-201 were washed in PSG, resuspended in rabbit anti-VSG-1 antiserum diluted 1:50 in PSG and incubated at 25 °C for 20 min. They were then washed in PSG, deposited on microscope slides, fixed and incubated with monoclonal mouse anti-VSG-201.

Bound antibodies were revealed as described above.

Stable expression of two variable surface glycoproteins by cloned *Trypanosoma equiperdum*

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African trypanosomes are thought to evade the host immune system by periodically changing their variable surface glycoprotein (VSG). VSG genes are activated by a complex process involving the duplicative transposition of silent basic copy genes to one of several expression sites¹⁻³. These expression-linked copies (ELCs) of the VSG genes are also subject to regulation within expression sites by as yet unknown mechanisms⁴⁻⁹. It is generally assumed that trypanosomes can express only one VSG gene at a time. Nevertheless, the finding that they contain multiple VSG gene expression sites suggests that multiple expression is possible. We show here that *Trypanosoma equiperdum* can stably express two VSG genes in a simple axenic culture system¹⁰ and that both antigens are present on the cell surface. The two antigens do not co-cap or form heterodimers. Their corresponding genes show no cross-hybridization and are situated in different telomere-linked expression sites. Northern blot analysis reveals that both genes are active in the double expressors.

BoTat-1 (Bordeaux trypanozoon antigen type-1)¹¹ trypanosomes were placed in axenic tissue culture as described previously¹⁰. This culture system allows long-term growth of bloodstream forms with generation times comparable to those observed *in vivo*. After various intervals, the cultured trypanosomes were examined using monoclonal anti-VSG-1 antibodies.

When 5-10% of the parasites no longer expressed VSG-1 on their surface, they were cloned in culture and a clone of non-VSG-1 trypanosomes, called BoTat-201, was isolated. Hybridomas producing anti-VSG-201 antibodies were produced as described elsewhere¹² and shown to react only with BoTat-201. Rabbit antibodies directed against purified VSG-201 were also produced. These antibodies specifically agglutinated BoTat-201 parasites and in immunofluorescence tests reacted specifically with BoTat-201. Both monoclonal and polyclonal antibodies were used for the analysis of variation in culture. Identical results were obtained with both. Cultures begun with BoTat-1 were examined for the presence of VSG-1- and VSG-201-bearing cells. Surprisingly, large numbers of cells expressing both antigens were found in the cultures.

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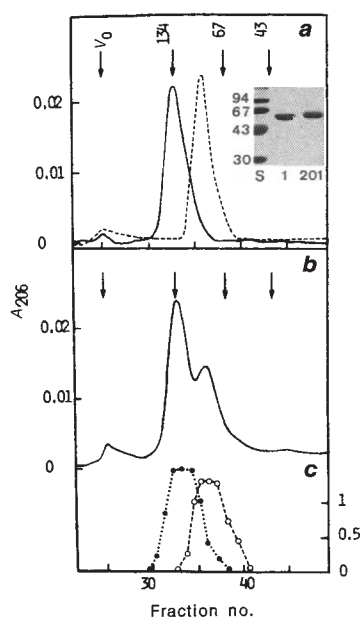


Fig. 2 Gel filtration of purified VSGs. VSGs were isolated from trypanosome populations by freezing and thawing in distilled water and chromatography of the soluble proteins on concanavalin A-Sepharose (Pharmacia)¹⁴. The eluted VSGs were applied to a 1.6×120 cm calibrated column of Sephacryl ACA44 and the column was developed with PBS at a flow rate of 18 ml h⁻¹. Numbered arrows represent M_r s of standards ($\times 10^{-3}$). **a**, VSG-1 (—) and VSG-201 (---) isolated from mono-expressing populations of trypanosomes. Inset, SDS-polyacrylamide gel of purified VSGs. M_r s of standards (S) are given ($\times 10^{-3}$). **b**, VSG purified from doubly expressing trypanosomes 2 days after inoculation into mice. The population consisted of 57% double expressors and 43% single expressors for VSG-1 (see Table 1). **c**, Aliquots of the fractions of **b** were assayed for their content of VSG-1 and VSG-201 by enzyme-linked immunosorbent assay¹⁶ using monoclonal mouse antibodies specific for each antigen. Aliquots (0.1 ml) were coated on the walls of microtitre wells (Nunc). Unbound antigen was removed by washing with 0.4% Tween 20 in PBS. Duplicate wells were then treated with 0.1 ml of either monoclonal mouse anti-VSG-1 (●) or anti-VSG-201 (○). After incubation for 2 h at 37 °C the wells were washed, and bound immunoglobulins revealed with peroxidase-conjugated sheep anti-mouse immunoglobulin (Pasteur) as described previously¹⁶.

To determine whether stable double expression could be maintained, the doubly expressing parasites were cloned in culture and then grown either *in vitro* or in mice. The cloned trypanosomes were examined at various intervals with the two antisera and the results are presented in Fig. 1 and Table 1. After 14 days (56 generations) in culture, more than 90% of the parasites still co-expressed both antigens. By 30 days (120 generations) only 16% expressed both antigens, the remainder expressing either VSG-1 or VSG-201. Thus, *in vitro*, double VSG expression is relatively stable and persists after cloning and long-term growth. In contrast, passage in mice resulted in the rapid loss of parasites expressing both antigens and the appearance of populations of pure VSG-1-expressing cells.

To verify that the putative double VSG expression was not due to cross-reactivity of anti-VSG-1 and anti-VSG-201 antibodies, or to the presence of a hybrid VSG, capping experiments were performed. Live parasites co-expressing both antigens were incubated at 25 °C for 20 min with rabbit anti-VSG-1 antibodies. After washing and fixation, the parasites were stained with monoclonal mouse anti-VSG-201 and washed further. Bound antibodies were revealed using the appropriate fluorescent anti-serum (Fig. 1). Whereas the VSG-1 antigen was localized in caps at the poles of the cells, the VSG-201 was evenly distributed



Fig. 3 **a**, Restriction maps of VSG-1 and VSG-201 gene ELCs in trypanosomes expressing both antigens and the cDNA clones used in the present study. cDNA cloning and restriction mapping were performed as described previously². Symbols: S, *Sall*; St, *Sst*I; H, *Hind*III; B, *Bam*HI; P, *Pst*I, *Rsa*I; R, *Eco*RI; Sa, *Sac*I; Xb, *Xba*I; Xh, *Xho*I; Pl, *Pvu*I; P2, *Pvu*II; D, *Dde*I; M, *Msp*I; A, *Ava*II; Ha, *Hae*III; A1, *Ava*I; Ah, *Aha*II; T, telomere. **b**, Northern blot analysis of poly(A) RNA from BoTat-1 (lanes 1, 4), trypanosomes expressing VSG-1 and VSG-201 (lanes 2, 5) and BoTat-201 (lanes 3, 6), probed with ³²P-labelled VSG-1-specific plasmid pTe1-612; lanes 4–6, probed with ³²P-labelled VSG-201-specific plasmid pTe201. The arrow indicates 2 kilobases. All methods were as described previously^{2,6,8}.

over the cells. This experiment definitively demonstrates that a single trypanosome can express both antigens and that each VSG behaves as an independent protein on the cell surface, thus eliminating the possibility of a hybrid protein.

Some VSGs are known to exist as dimers in solution and it has been suggested that they exist as such on the surface of the trypanosomes¹³. However, our co-capping experiments suggested that no heterodimers exist on the surface of the doubly expressing parasites. To confirm and extend this observation, variant antigen was purified from doubly expressing parasites and analysed by gel filtration chromatography¹⁴. Figure 2 shows that the material eluted in two peaks. The first peak, with an apparent relative molecular mass (M_r) of 134,000, corresponded to pure VSG-1 antigen, while the second peak, with an apparent M_r of 75,000, corresponds to VSG-201 antigen. While VSG-1 has a M_r of 58,000 when analysed by polyacrylamide gel electrophoresis in the presence of SDS and reducing agents, VSG-201 has an apparent M_r of 62,000. Non-denatured VSG-1 behaves like a dimer whereas VSG-201 migrates at a position closer to that expected of a monomer than of a dimer. Other methods of antigen extraction have given the same results, suggesting that VSG-1 is a dimer on the cell surface whereas VSG-201 may be a monomer. The finding that there were no heterodimers of VSG-1 and VSG-201 further confirms that the two antigens behave independently on the cell surface.

We have isolated complementary DNA clones corresponding to VSG-201. The cDNA clone pTe201 was used to determine the restriction map of the VSG-201 gene ELC, which is shown together with that of the previously characterized VSG-1 ELC from BoTat-1 (Fig. 3a)². Note that the VSG-201 gene is in the

Table 1 Stability of double expression in tissue culture and in mice

Days of culture	Generations	% Expression of VSG		
		1+201	1	201
<i>In vitro</i>				
7	28	98	1	1
14	56	93	4	3
30	120	16	40	44
<i>In mice</i>				
	0	>95		
2	8	57	40	3
3	12	8	91	1

The doubly expressing trypanosomes were cloned and cultured *in vitro*, and the cultures diluted with fresh medium daily. On day 9 of culture, 10⁷ parasites were inoculated into a mouse and, after 2 days, were passaged into a second mouse. At the indicated times the parasites were examined by immunofluorescence for expression of VSG-1 and VSG-201 as described in Fig. 1 legend.

previously characterized 1.78 expression site¹ and is activated without apparent duplication, as has been demonstrated for several variant antigen genes of *Trypanosoma brucei*^{4,6,8,15}.

RNA from pure VSG-1, pure VSG-201 and cloned doubly expressing trypanosomes was analysed with ³²P-labelled cDNA probes in Northern blots. Figure 3b shows that there is no cross-hybridization between the two RNAs and that both are present in the double expressors.

Our results demonstrate that African trypanosomes are able to express two VSGs on their surface at the same time. This double expression is relatively stable, as the parasites can be cloned and grown for over 100 generations *in vitro* presenting both antigens. It is clear that if the relatively stable expression of the two antigens reported here were to occur *in vivo*, the doubly expressing parasites would probably not be seen, as they would be killed by antibodies directed against the antigen expressed first. Also, our results (Table 1) demonstrate that trypanosomes expressing both VSG-1 and VSG-201 are rapidly lost from the population in mice, where immune selection is negligible. However, it is possible that *in vivo* double expression is a transient phase of the process of antigenic variation.

Although double antigen expression is relatively stable *in vitro*, the population slowly drifts to single expression, with no bias towards either antigen. On the other hand, when the double expressors are inoculated into mice, there is a rapid evolution towards single expression of VSG-1. Although variant antigen type (VAT)-201 grows as rapidly as VAT-1 *in vivo* when adapted to growth in the mouse, it requires a period of adaptation when transferred from culture to the mouse. This is not the case for VAT-1, which grows rapidly when transferred from culture to mouse (ref. 11 and G. Thon, unpublished). It is thus probable that, when the double expressors were injected into mice, the VAT-1s outgrew the double expressors and the VAT-201s.

The extent to which the double expression described here is specific to the two VSG genes examined is not known. We have found that another antigen, VSG-202, is co-expressed with VSG-1 in trypanosomes that have lost the VSG-201 gene (unpublished results). Double expression therefore seems to be a reasonably common phenomenon.

The two VSG genes described here and shown to be co-expressed occur in different expression sites. This demonstrates that each expression site can be regulated independently of the others and that activation of one site is not mutually exclusive of activation of others. Our results thus exclude certain models for the mechanism of variant antigen gene regulation, such as those which propose the presence of a single mobile regulatory element that moves from one expression site to another. Our findings also indicate that trypanosomes express only one antigen *in vivo* because of a selection against double antigen expression in the animal rather than because of an intrinsic genetic mechanism in the parasite.

Finally, the finding of double VSG gene expression raises questions about the functional significance of the cassette-like mechanism for VSG gene regulation. The most obvious rationale for such a mechanism is to guarantee the expression of one antigen at a time. However, this cannot be its role in *T. equiperdum*, for there are multiple expression sites and we have demonstrated here that a single trypanosome can express at least two antigen genes.

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Nucleotide sequence of chicken *c-myb* complementary DNA and implications for *myb* oncogene activation

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Avian myeloblastosis virus (AMV), like other acute transforming viruses, arose by recombination between its helper virus and host cellular sequences. The latter sequences, termed *v-myb*, are responsible for the oncogenic properties of the virus. AMV causes acute myeloblastic leukaemia in chickens and transforms a specific class of haematopoietic cells *in vitro*, but does not induce morphological transformation of cultured fibroblasts, suggesting that only a restricted target-cell population is responsive to its transforming gene product^{1,2}. The normal cellular counterpart of *v-myb*, *c-myb*, is highly conserved and is present in all vertebrate and some invertebrate species examined^{3,4}. DNA rearrangements and altered expression of the *myb* oncogene have been reported in mouse lymphoid tumours⁵⁻⁷ and human myeloid⁸ and colon tumours⁹. The mechanism of activation of the cellular proto-oncogenes is thought to involve the structural alteration of the coding regions that result in either the synthesis of an altered gene product or the enhanced expression of a proto-oncogene caused by alterations in its regulatory elements. To distinguish between these two mechanisms, we have cloned and sequenced the chicken *c-myb* complementary DNA and compared it with that of *v-myb* sequences. We demonstrate that during the transduction of the cellular sequences and/or viral passage a substantial portion of the coding region of the *c-myb* gene has been lost from both the 5' and 3' ends, resulting in the generation of a truncated gene product that mediates the transforming function of the virus.

Before cloning the normal *c-myb* cDNA sequences, we screened messenger RNA preparations from several organs of chicken and found that the proto-oncogene is expressed in substantial amounts in thymus and bursa as a 4.0-kilobase (kb)-long mRNA. Thus, for construction of a cDNA library containing the *myb* gene, thymuses from ~50 chickens were pooled and the RNA extracted as described previously⁵. After two cycles of purification on oligo(dT)-cellulose, the mRNA was used to prepare cDNA as described in Fig. 1 legend. The restriction map of the chicken *c-myb* cDNA clone that was used for sequence analysis is shown in Fig. 1. Figure 2 shows the nucleotide sequence of the coding region of this cDNA clone. The longest open reading frame extends from nucleotides 13 to 2,163 and contains an initiator codon, ATG, at position 63-65. This seems to be the true initiator codon because a terminator codon is present in-frame with this ATG at position 13-15. Furthermore, this ATG is in the sequence CCGTCCATGG which is similar to the consensus sequence CC(A/G)CCATGG for translation initiation as defined by Kozak¹⁰. The open reading frame shown in Fig. 2 could encode a polypeptide of 699 amino acids with an estimated relative molecular mass (*M_r*) of 77,000 (77K), in good agreement with the estimated size of 75K for the *c-myb*-encoded protein¹¹. This open reading frame is

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preceded by ~500 base pairs (bp) of noncoding sequences at the 5' end and followed by a stretch of 1,400 bp of noncoding sequences at the 3' end.

Analysis of the predicted *c-myb* amino-acid sequence in a dot-matrix program¹² reveals a stretch of three tandem repeats in the amino-terminal end of the molecule (Fig. 3). These repeats occur in amino-acid positions 96-147, 148-199 and 200-250, the first two repeats containing more homology than the third. The role of these repeats is unknown. However, as the *myb* protein accumulates in the nucleus¹³⁻¹⁵ and also seems to bind to DNA¹⁵, these repeats could be involved in DNA binding or nuclear localization. A comparison of these sequences with those of mouse *c-myb*¹⁶ shows that this region is highly conserved between the two species. Thus, it seems probable that the region encodes a critical function.

A comparison of the cDNA sequence with that of the *v-myb* sequences of AMV^{17,18} reveals that the two genes are homologous from nucleotides 450 to 1,563 (Fig. 2). Sequences homologous with *v-myb*-E26 (oncogene of the E26 virus)¹⁹ are contained in nucleotides 478-1,341. These comparisons suggest features that may be required for retroviral activation of this oncogene. In both cases similar stretches of amino- and carboxy-terminal sequences have been deleted. The AMV and E26 proteins are 142 and 150 amino acids shorter, respectively, than the normal protein at their amino-terminal ends, and 192 and 272 amino acids shorter at their carboxy-terminal ends. In AMV-transformed myeloblasts, these deletions cause the truncation of the *v-myb* gene product with a resulting *M_r* of 45K (refs 11, 20). The amino-terminal deletions result in the loss of the first

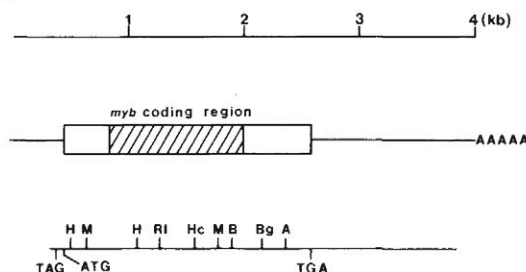


Fig. 1 Analysis of the coding region of the 4.0-kb chicken *myb* mRNA, performed on a single phage ~3.5-kb cDNA clone spanning the entire region. The cDNA library was constructed from poly(A) RNA isolated from chicken thymus. The first and second strands were synthesized by a modification of the method of Gubler and Hoffmann²². First-strand synthesis was accomplished using oligo(dT) primer and M-MuLV reverse transcriptase (Bethesda Research Laboratories) under conditions recommended by the manufacturer. Second-strand synthesis was performed exactly as described elsewhere²² using RNase H and *Escherichia coli* PolI. The cDNA was then methylated with *E. coli* RI methylase and treated with T4 polymerase to blunt the ends of the cDNA before ligation to *Eco*RI linkers. After addition of linkers, the cDNA was digested with *Eco*RI and the digested linkers removed by four rounds of precipitation with 0.15 vol. of 3 M sodium acetate and 0.6 vol. of isopropanol. 200 ng of cDNA was ligated to 1 µg of λ gt10 arms to obtain a library of 4×10^6 recombinants. We obtained ~20 *v-myb*-hybridizing clones, one of which (clone 7) contained the entire *myb*-coding region within a 3.5-kb insert. Restriction sites shown are: H, *Hae*II; M, *Mst*II; R, *Eco*RI; Hc, *Hinc*II; B, *Bgl*I; Bg, *Bgl*II.

TTCCGGCGGC	CGTAGCGGGC	CATCTGCACG	CGGAGCTCGC	GGCCATCCAG	CACGGCCCCG	TCC	30
ATG GCG TCC ATC GCG TCC TCG GCG TCG CGT TTG TCG TGG AAA CGG ACG AAT GCG	100						
MET Ala Ser Ile Ala Ser Ser Ala Ser Arg Leu Ser Trp Lys Arg Thr Asn Ala	150						
AAC CCG CGG CTC CTC GGC ACT TCG CTG CGC GGC GGC GGA GCG CCT GGG CGC AGC	200						
Asn Pro Arg Leu Leu Gly Thr Ser Leu Arg Gly Gly Ala Pro Gly Arg Ser	250						
CCG GCA GCA GGG CGC GGC GGC GGC GGC GGC CTC CCC CGT CCT CTT CCC CCA GCG	300						
Pro Ala Ala Gly Arg Gly Gly Gly Leu Pro Arg Pro Leu Pro Pro Ala	350						
GCC GCC GCG AGG ATG GCG AGC AGC CCC GGC CAC AGC ATA TAC AGC AGC GAT GAC	400						
Ala Ala Ala Arg MET Ala Arg Arg Pro Arg His Ser Ile Tyr Ser Ser Asp Asp	450						
GAT GAA GAA GAT GTT GAT ATG TAC GAC CAC GAT TAC GAC GGC CTG CTT CCT AAG	500						
Asp Glu Glu Asp Val Glu MET Tyr Asp His Asp Tyr Asp His Asp Tyr Asp His	550						
GCT GGG AAA CGT CAC CTA GGG AAA ACC AGG TGG ACC CGT GAA GAG GAT GAG AAA	600						
Ala Gly Lys Arg His Leu Gly Lys Thr Arg Trp Thr Arg Glu Glu Asp Glu Lys	650						
CTG AAG AAA CTT GTG GAA CAG AAT GGC ACA GAA GAG TGG AAA GTT ATT GCC AGT	700						
Leu Lys Leu Val Glu Glu Lys Asn Gly Thr Glu Asp Trp Lys Val Ile Ala Ser	750						
TTC CTT CTT AAT CGG ACA GAT GTT CAG TGC CAG CAC CGG TGG CAG AAT GTA TTA	800						
Phe Leu Pro Asn Arg Thr Asp Val Gln Cys Gln His Arg Trp Gln Lys Val Leu	850						
AAC CCA GAA CTT ATC AAA GGT CAC TGG ACT AAA GAG GAG GAT CAA AGG GTA ATA	900						
Asn Pro Glu Leu Ile Lys Gly Pro Trp Thr Thr Glu Glu Asp Gln Arg Val Ile	950						
GAA CTC GTG CAG AAA TAC GGT CCA AAG CGC TGG TCG GTC ATT GCT AAG CAT TTG	1000						
Glu Leu Val Lys Lys Tyr Gly Thr Lys Arg Trp Ser Val Ile Ile Thr His Leu	1050						
AAG GGA AGG ATT GGA AAA CAG TGC AGG GAG AGG TGG CAC AAC CAT CTG AAT CCA	1100						
Lys Gly Arg Ile Gly Lys Gln Cys Arg Glu Arg Trp His Asn His Leu Asn Pro	1150						
GAA GTG AAG AAA ACC TCC TGG ACA GAA GAG GAA GAT AGA ATT ATT TAC CAG GCA	1200						
Glu Val Lys Lys Thr Ser Trp Thr Glu Glu Asp Arg Ile Ile Thr His Leu	1250						
CAC AAG AGA CTG GGA AAC AGA TGG GCA GAA ATT GCA AAG TTG CTG CCT GGA CGG	1300						
His Lys Arg Leu Gly Asn Arg Trp Ala Glu Ile Ala Lys Leu Pro Gly Arg	1350						
ACT GAT AAC GCT ATC AAG CAC TGG AAT TCC ACC ATC CGC GGC AAG GTC GAG	1400						
Thr Asp Asn Ala Ile Lys Asn His Trp Asn Ser Thr MET Arg Arg Lys Val Glu	1450						
CAG GAG GGT TAC CTG CAG GAG TCC TCC AAA GCC GGC CTG CCC TCG GCA ACC ACC	1500						
Gln Glu Gly Tyr Leu Lys Ser Ser Lys Ala Glu Lys Thr Ala Thr Thr	1550						
GGC TTC CAG AAG AGC AGC CAC CTG ATC GGC TTT GCC CAC AAC CCA CCT GCA GGC	1600						
Gly Phe Gln Lys Ser Ser His Leu MET Ala Phe Ala His Asn Pro Pro Ala Gly	1650						
CCG CTC CCG GGC GGC GGC CAG GGC CCG CTG GGC AGT GAC TAC CCA TAC TAC CAC	1700						
Pro Leu Pro Gly Ala Gly Gln Ala Pro Leu Gly Ser Asp Tyr Pro Tyr Tyr His	1750						
ATT GCT GAG CCA CAA AAT GTC CCT GGT GAG ATC CCA TAT CCA GTA GCA CTG CAT	1800						
Ile Ala Glu Pro Gln Asn Val Pro Gly Lys Ile Pro Tyr Pro Val Ala Leu His	1850						
GTA AAT ATT GTC AAT GTT CCT CAG CCA GCT GCT GCA GCT ATT CAG AGA CAC TAT	1900						
Val Asn Ile Val Asn Val Pro Gln Pro Ala Ala Ala Ile Gln Arg His Tyr	1950						
AAT GAT GAA GAC CCT GAG AAA GAA AAA CGA ATA AAG GAA TTA GAG TTG CTA CTT	2000						
Asn Asp Glu Asp Pro Glu Thr Lys Arg Ile Lys Glu Leu Glu Thr Leu Leu	2050						

Fig. 2 The nucleotide sequence of the coding region of the *c-myb* cDNA clone, obtained using the method of Maxam and Gilbert²³. The amino-acid sequence predicted for the proto-oncogene product is shown below the nucleotide sequence. Pertinent restriction enzyme sites and *v-onc*-homologous regions are shown. The two terminator codons in-frame with the reading frame upstream and downstream are indicated by asterisks. Arrows indicate restriction enzyme cleavage sites or beginning and end of *v-myb*-homologous sequences.

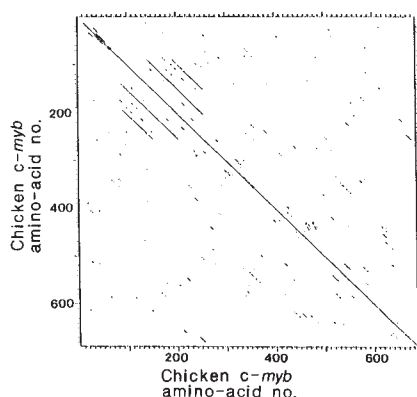


Fig. 3 Dot-matrix analysis for internal repeats within the chicken *c-myb* coding region. Segments of 25 amino acids were compared sequentially with each 25-amino-acid-long segment of the protein. A dot was placed on the matrix at the appropriate position when the total mutation data matrix score for the comparison was ≥ 20 .

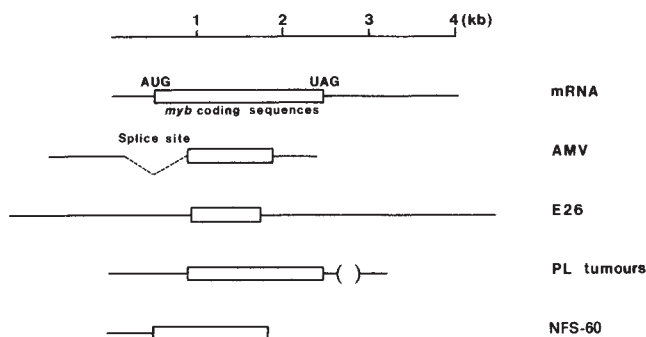


Fig. 4 Structural comparison of the *myb*-coding region of chicken *c-myb* mRNA (normal) with activated forms of *myb* mRNAs in the AMV and E26 viruses and PL and NFS-60 tumour cells. Boxes represent *myb*-coding sequences; straight lines represent flanking viral (AMV and E26) or cellular (PL tumours and NFS-60) sequences. The parentheses in PL tumour RNA indicate rearrangements at the 3' end which have not yet been precisely defined.

analogous to that found in AMV (S. Lavu and E.P.R., in preparation) (Fig. 4).

A second example of activation of murine *c-myb* is seen in the NSF-60 tumour cell line induced by Cas-Br-M-MuLV²¹. In this myeloid tumour cell line the integration of the provirus has occurred at the 3' end of the *myb* locus, which results in the synthesis of a truncated mRNA. We have recently mapped the point of integration of this virus to the point that corresponds to nucleotide 1,425 in the *c-myb* sequence presented in Fig. 2. This suggests that the *myb* protein produced in these tumour cells contains a carboxy-terminal deletion similar to that found in the AMV and E26 *myb* proteins.

Our DNA sequence comparison shows that the normal *myb* protein contains additional amino acids at the N-terminal and/or the C-terminal end when compared with any of the activated forms of this protein. The structural changes that occur as a result of these deletions probably contribute to the oncogenic potential of the proteins. The availability of chicken *c-myb* cDNA clones will allow us to make various retroviral constructs directly to test our hypothesis.

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of the three tandem repeats in the two *v-myb* proteins which may also be relevant for transformation.

In addition to the avian systems which suggest that deletions are important in the activation of *myb*, two other systems support this hypothesis. Analysis of the rearranged mouse *c-myb* genes in the Abelson plasmacytoid lymphosarcomas (ABPLs) induced by a mixture of Abelson murine leukaemia virus and Moloney murine leukaemia retrovirus (M-MuLV), reveals that the genomic insertion of the virus occurs in the intron immediately upstream of the sequences corresponding to nucleotide 450 in the chicken cDNA clone. This results in a deletion of the amino-terminal sequences in the aberrant *c-myb* transcripts

Corrigendum

Major shear zones and autochthonous Dalradian in the north-east Scottish Caledonides

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